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SOME FACTORS WHICH AFFECT THE DIASTATIC ACTIVITY IN WHEAT [1]

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Introduction

The low gassing rate of flour from the last few crops, especially in the Southwest, has stimulated much interest in the diastatic activity of wheat and flour. It has been found that by mixing small amounts of flour from malted wheat with such flour, better loaf volume and crust color can be obtained.

Several methods are in use for testing flour either for diastatic activity or gassing power. Gassing rate or power may be estimated either by measuring the pressure or the amount of gas produced by the fermentation of a suspension of yeast and flour, or by measuring the amount of gas production from dough. Diastatic activity is usually estimated by determining the milligrams of maltose produced when a flour-water suspension is digested at 30° C. for one hour (Blish and Sandstedt, 1933). Davis and Worley (1934) have observed that a close relationship exists between diastatic activity and gassing ability or power. The amount of maltose or sugar already present in the wheat or flour is determined by adding the sodium tungstate before the digestion so as to stop the enzyme action. The baking test with appropriate modification may also be used to indicate deficiency of diastase in flour.

In Europe the gassing rate has been considered of importance for many years because of the usual absence of sugar from the dough formula. Flours milled from Western European wheats are as a rule such good "gassers" that when blended with the poorer "gassers" of most imported wheats an adequate quantity of gas is produced.

Historical

Wood (1907) showed that the rate of gas production from a flour is closely related to the size of the loaf and also to the general baking

[1] Contribution No. 47, Department of Milling Industry.

value as indicated by bakers' marks which were based both on size and shape of the loaf. Wood mentions that Maurizio (Landw. Jarb. XXXI, 1902) had shown that without sugar there were greater differences in the loaf sizes than when sugar was added to the dough. The importance of gas retention was also shown in Wood's paper since this also determines the size of the loaf as well as the amount of gas evolved.

Two-fifths to one-third of the starch in flour may be converted into sugars by simply digesting in water at a suitable temperature for one hour according to Swanson and Calvin (1913). These authors observed the greatest amololytic activity at 65° C. It has since been shown that the optimum is near 63.5° C. They also investigated the effects of the addition of small amounts of acid, but obtained no notable results, probably because the digestion was made at a temperature at which the maximum transformation took place. Important contributions to the knowledge of enzymes in flour were made by Baker and Hulton (1908) and Ford and Gutrie (1908). Sörenson (1909) had shown the importance of controlling the hydrogen-ion concentration in connection with enzyme studies.

Instead of trying to obtain the maximum diastatic activity, as had been done by Swanson and Calvin, Rumsey (1922) endeavored to approximate the temperature used in dough fermentation by digesting flour in water for one hour at 27° C. Rumsey also improved the technique and showed the importance of the hydrogen-ion concentration whose optimum he found to be pH = 4.7. He also found that flours from different wheats varied considerably in diastatic activity; patent flours from hard red spring wheats having the highest diastatic activity, and flour from white wheat had the lowest.

Grewe and Bailey (1927) determined the diastatic activity of 17 flours both at the initial hydrogen-ion concentration which varied between pH = 5.52 and pH = 6.39, and at a constant hydrogen-ion concentration of pH = 5 produced by the addition of lactic acid. The figures for diastatic activity expressed in terms of milligrams of maltose produced per 10 g. of flour were less than 100 in the four flours exhibiting the weakest diastatic activities. These included: a straight Pacific Coast soft wheat flour, and three soft winter wheat flours. The corresponding figures for the four flours with the strongest activity were over 200 mg. Three of these were from hard spring wheat and one from a mixture of hard winter and hard spring. Raising the hydrogen-ion concentration to pH = 5 increased the diastatic activity in every case, however, not in exact proportion to the amount of lactic acid required.

Mangels (1926) found that the diastatic activity of straight grade flour from different varieties of hard red spring wheat grown in the

same location varied from 66.5 mg. in Marquis to 126.9 mg. in Kota. Mangels also found that the cropping system and the fertilizers added apparently influenced the diastatic activity in Ceres wheat grown at Fargo.

Sherwood and Bailey (1926) showed that the diastatic activity of flour may be greatly increased by adding germinated wheat to the mill mixture. Olson (1917) found that wheat which had been germinated had from 7 to 9 times the diastatic activity of non-germinated wheat.

Grewe and Bailey (1927a) observed no correlation between the size of starch granules in wheat flour and the diastatic activity. Shollenberger and Coleman (1926) separated flour into various sized granules by sifting and found a diastatic activity, measured in milligrams maltose, of 268.5 for the portion that had passed 12XX and was retained by 16XX; 423.3 in the portion which passed 20XX and was retained by 25XX; and 509 in the portion which had passed 25XX. They also found that regrinding a number of times increased the diastatic activity.

Pascoe, Gortner, and Sherwood (1930) reported that the diastatic activity of commercially milled patent flour was about twice that of experimentally milled flour. They also found that long grinding in a ball mill materially increased the diastatic activity. The mill stream flours differed greatly in their diastatic activity, being weakest in the third break flour and strongest in sizings. These investigators believed that the saccharogenic activity is particularly indicative of the germ content of the various streams. That commercially milled flours have a much higher diastatic activity than flours experimentally milled from the same wheat mix was also found by Sandstedt and Blish (1933).

The diastatic activity of the various classes of wheat, and of several other cereal grains have been studied by Coleman, Snider, and Dixon (1934). These authors also investigated the influence of the preparation of the samples. The hard red spring wheats had a higher range than the hard red winter or the soft red winter, while the durum wheats had the highest. Markley and Bailey (1934) studied the effect of milling methods and tempering upon diastatic activities of commercially milled and the experimentally milled flour as well as the finely ground whole wheat meal. The mean of the experimentally milled flour was 168.89, of the commercially milled 250, and of the whole wheat meal 270.21 Rumsey units.

That merely keeping wheat moist enough for germination does not increase the sugar content unless the process of germination actually starts or takes place, was shown by Swanson, Fitz, and Dunton (1916). Wheat with a sugar content of 2.23% was kept moist enough for germination, but failed to do so because the temperature of the room was too high, had after 4 days a sugar content of 1.89%, after which the

sugar content remained practically constant. In another experiment in which the temperature conditions were favorable to germination, the sugar content of the wheat at the end of 4 days was 2.56% and after 7 days 3.93%.

That the amount of moisture added in long tempers will not increase the sugar content of flour, was shown by Swanson (1934). Wheat was stored under various conditions of temperature and moisture for various lengths of time, and it was found that the diastatic activity was not increased by high moisture; on the contrary the diastatic activity was greatest in the samples stored with the low moisture. The increased respiration, caused by the addition of tempering water, evidently consumed the sugar faster than it was formed.

Experimental [2]

Influence of Granulation of the Sample

Several preliminary experiments were conducted to determine the effect that fineness of grinding the sample has upon the diastatic activity. The size of the particles was found to exert a decided influence, however, grinding finer than to pass 60 mesh wire screen seemed to serve no useful purpose, hence the samples in the following experiments were ground to this fineness in a modified coffee mill belted for power. The mill was so arranged that no mechanical losses took place and no appreciable heating was observed. After each grinding the sample was sifted and the portion which did not pass through the sieve was reground.

Diastatic Activity as Related to Varieties

Varieties Grown in Kansas

A number of wheat varieties grown at Manhattan, Hays, and other points in the state are tested each year for milling and baking qualities. Samples of these wheats were used for testing the variations in diastatic activity that may occur among different varieties grown in the same locality, and also among the same varieties grown in different localities. The method of Blish and Sandstedt (1933) was used in all the determinations, and the results are recorded in all the tables as milligrams maltose produced per 10 g. ground material, and are usually arranged according to increasing values. The data recorded in Table I are the results secured in a comparison of the diastatic activity of varieties of wheat grown at Manhattan. Varieties grown under the same conditions of climate and soil show a variation from 187 to 268. The soft wheat

[2] The author desires to express his appreciation to Mr. J. Forrest Wolf for efficient assistance in making the determinations for these investigations.

TABLE I

DIASTATIC ACTIVITIES OF VARIETIES OF WHEAT GROWN AT MANHATTAN, 1933

Milligrams of maltose per 10 g. of wheat-meal or flour

Serial number	Variety	Maltose		Serial number	Variety	Maltose	
		Wheat	Flour			Wheat	Flour
		Mg.	*Mg.*			*Mg.*	*Mg.*
18920	Minturki	187	72	18921	Oro	235	103
18925	Clarkan	187	86	18909	Kanred × Hard Federation	237	88
18926	Harvest Queen	189	73	18907	Kanred × Hard Federation	239	100
18914	Blackhull	191	76	18931	Bulk Hybrid Selection H. 519	240	110
18924	Fulcaster	204	76	18923	Kawvale	242	116
18912	Tenmarq 514	207	96	18916	Turkey	243	99
18913	Tenmarq 2670	209	120	18918	Hays No. 2	244	85
18915	Cheyenne	216	87	18929	Bulk Hybrid Selection H. 517	244	98
18922	Cooperatorka	216	121	18930	Bulk Hybrid Selection H. 518	245	115
18911	Kanred × Marquis	222	78	18908	Kanred × Hard Federation	256	94
18905	Early Blackhull	223	80	18904	Kanred	258	96
18910	Kanred × Marquis	225	79	18906	Quivira	268	119
18917	Kharkoff	227	91	18927	P × K	268	106
18919	Nebraska 60	234	89	18928	P1066 × Prelude	268	122

varieties gave as a rule lower values than the hard varieties. The values secured from the flours milled on an Allis experimental mill, are considerably less than half those obtained on wheat, and the correlation with the wheat values is not very high.

Some of the same varieties were also grown at Hays, Kansas, and the diastatic activity of these is given in Table II. The maltose value from each wheat grown at Hays was less than the amount obtained from the same variety grown at Manhattan. On the other hand, the flours milled from the wheats grown at Hays were somewhat higher

TABLE II

DIASTATIC ACTIVITIES OF VARIETIES OF WHEAT GROWN AT HAYS, 1933

Milligrams of maltose per 10 g. of wheat-meal or flour

Serial number	Variety	Maltose	
		Wheat	Flour
		Mg.	*Mg.*
18979	Blackhull	168	100
18984	Early Blackhull	168	110
18985	Oro	173	100
18980	Tenmarq	181	105
18983	Kanred x Hard Federation	185	130
18978	Cheyenne	198	102
18982	P1066-1 x Burbank	200	116
18976	Kharkof	206	90
18981	Quivira	207	121
18977	Turkey	215	92

in their diastatic activity than those milled from wheats grown at Manhattan. Thus, the drier climate apparently has an influence upon this characteristic. However, the 1933 season at Manhattan was very dry.

The results secured from varieties grown in cooperation with farmers in various parts of Kansas are given in Table III. The values are

TABLE III

DIASTATIC ACTIVITIES OF WHEATS GROWN IN DIFFERENT LOCALITIES IN KANSAS, 1933. *Milligrams of maltose per 10 g. of wheat-meal or flour*

Serial number	Variety	Production area	Maltose		Serial number	Variety	Production area	Maltose	
			Wheat	Flour				Wheat	Flour
			Mg.	*Mg.*				*Mg.*	*Mg.*
19021	Clarkan	—	165	82	19027	Turkey	S.W.	224	136
19020	Fulcaster	—	183	98	19012	Tenmarq	N.W.	236	109
19023	Cooperatorka	—	223	101	19016	Tenmarq	S.W.	256	172
19019	Kawvale	—	226	130	19013	Cheyenne	N.W.	243	102
19029	Early Blackhull	N.W.	216	—	19017	Cheyenne	S.W.	253	154
19026	Early Blackhull	S.W.	205	115	19011	Kanred	N.W.	243	121
19025	Blackhull	N.W.	208	—	19015	Kanred	S.W.	248	117
19028	Blackhull	S.W.	210	—	19014	Quivira	N.W.	268	121
19024	Turkey	N.W.	252	124	19018	Quivira	S.W.	320	158

somewhat higher than were obtained from the same varieties grown at Hays, as shown in Table II. Again, the soft wheats gave lower values than the hard wheat varieties. Slight differences were exhibited in the diastatic activity of the grain of a variety grown in northwest or in southwest Kansas, but the flour milled from a variety grown in southwest Kansas gave, in most cases, higher values. It appears from this that climatic factors have greater effect on the diastatic condition in the endosperm than of the kernel as a whole.

Samples of grain of the new varieties Tenmarq and Kawvale, grown in various localities in Kansas, were available and their diastatic activity was tested in order to obtain additional data relative to the influence of climatic factors upon diastatic variability. The results are given in Table IV.

The values for Tenmarq vary from 234 to 304, and on the whole are higher than for the varieties recorded in Tables I, II, and III, indicating that Tenmarq as a variety has a tendency to be high in diastatic activity. One of the parents of Tenmarq is Marquis, a spring wheat, and spring wheats are usually higher in diastatic activity. This indicates the possibilities of Tenmarq having inherited high diastatic activity from Marquis.

Kawvale averages somewhat higher than Tenmarq in its diastatic activity. The data in Tables I, II, and III indicate that soft wheats are somewhat less active in this respect than the hard varieties. Kawvale, however, is not a typical soft wheat; in milling characteristics it

TABLE IV

DIASTATIC ACTIVITY OF TENMARQ AND KAWVALE WHEAT GROWN IN VARIOUS LOCALITIES IN KANSAS, 1933. *Milligrams of maltose per 10 g. of wheat-meal or flour*

Serial number	Variety	Production area	Maltose	Serial number	Variety	Production area	Maltose
			Mg.				*Mg.*
19243	Tenmarq	Anthony	234	19222	Tenmarq	Riley	279
19231	"	Wichita	235	19225	"	Morrill	279
19244	"	Harper	244	19226	"	Morrill	284
19228	"	Harper	246	19215	"	Enterprise	290
19230	"	Hopewell	246	19216	"	Caldwell	301
19220	"	McPherson	247	19218	"	Belle Plaine	303
19233	"	Hutchinson	258	19214	"	Keats	304
19248	"	Winfield	264	19334	Kawvale	Whiting	264
19223	"	Luray	269	19339	"	Enterprise	272
19217	"	Hays	270	19238	"	Louisburg	283
19247	"	Culver	270	19241	"	Bucyrus	296
19219	"	Whitewater	272	19242	"	Humboldt	300
19236	"	Manhattan	276	19232	"	Easton	301
19224	"	New Cambria	277	19235	"	Ottawa	312
19221	"	Claflin	278	19240	"	Columbus	315
19229	"	Garfield	278				

behaves as a hard wheat, and in baking it has not been found to have the characteristics of a typical soft wheat. The higher results for Kawvale, shown in Table IV, may be partly due to climatic conditions, as most of these samples were grown further east than the Tenmarq samples.

Varieties Grown in Other States

Besides the varieties of wheat grown in Kansas a number of samples were obtained of varieties grown in other states and Canada. The data obtained from diastatic studies on the soft red winter and white wheats of this group are given in Table V. Where more than one sample of a variety was studied, the results are grouped according to increasing amounts of maltose found. The largest amounts were obtained from the white wheat samples, however, the locality apparently had a greater influence upon diastatic activity than the variety. The results from the varieties of which only one sample was available are arranged in the second column according to increasing amounts of maltose produced. There is a spread of 264 mg. from the lowest to the highest, but there is no apparent correlation between diastatic activity and location of growth.

The diastatic activity values obtained on hard spring wheats and three durum wheats are given in Table VI. The outstanding points to be observed in these results are the large variations within the varieties. The three durum samples gave as an average very much higher

TABLE V

DIASTATIC ACTIVITY OF SOFT RED WINTER AND WHITE WHEATS GROWN IN OTHER STATES

Milligrams of maltose per 10 g. of wheat-meal or flour

Serial number	Variety	Crop year	Production area	Maltose	Serial number	Variety	Crop year	Production area	Maltose
				Mg.					*Mg.*
18750	Harvest Queen	1932	Missouri	210	19303	American Banner	1933	New York	260
18754	" "	1932	Illinois	228	18781	Dawson's Golden Chaff	1932	Canada	260
18769	Fulcaster	1932	Pennsylvania	237	19278	Forward	1933	New York	275
18755	"	1932	Illinois	242	19275	Thrumbul	1933	Ohio	275
18752	"	1932	Missouri	264	19302	Bald Rock	1933	Michigan	287
19272	"	1933	Missouri	345	19276	American Banner	1933	Ohio	290
19274	Red Rock	1933	Ohio	305	19295	Hybrid	1933	Washington	302
19300	" "	1933	Michigan	310	19277	Fulhio	1933	Ohio	305
18779	Baart	1932	Colorado	284	19299	Hard Federation	1933	Oregon	310
18775	"	1932	California	293	19296	Albit	1933	Washington	315
19269	"	1933	California	420	19279	Junior No. 6	1933	New York	312
19298	Federation	1933	Oregon	258	19273	Michigan Wonder	1933	Missouri	314
19297	"	1933	Washington	320	19301	Berkley Rock	1933	New York	360
19270	"	1933	California	474	19271	Onas	1933	California	376
					19280	Valprize	1933	New York	390

TABLE VI

DIASTATIC ACTIVITIES OF HARD SPRING AND DURUM WHEATS

Milligrams of maltose per 10 g. of wheat-meal or flour

Serial number	Variety	Crop year	Production area	Maltose	Serial number	Variety	Crop year	Production area	Maltose
				Mg.					*Mg.*
19334	Marquis	1933	Leeds, N. Dak.	210	18720	Ceres	1933	Minnesota	345
19331	"	1933	Fessenden, N. Dak.	218	19286	"	1933	North Dakota	420
19341	"	1933	Ft. Benton, Mont.	244	19290	"	1933	Montana	555
19337	"	1933	Fargo, N. Dak.	262	19336	Reward	1933	Leeds, N. Dak.	211
19346	"	1933	Morris, Minn.	279	19339	"	1933	Fargo, N. Dak.	216
19282	"	1933	Minnesota	305	19333	"	1933	Fessenden, N. Dak.	256
19292	"	1933	South Dakota	335	18735	Garnet	1932	Saskatchewan, Canada	352
19285	"	1933	North Dakota	355	18748	"	1932	Alberta, Canada	363
19291	"	1933	Montana	482	18739	"	1932	Ottawa, Canada	376
19332	Ceres	1933	Fessenden, N. Dak.	239	19343	Thatcher	1933	Ft. Benton, Mont.	244
19338	"	1933	Fargo, N. Dak.	242	19340	"	1933	Fargo, N. Dak.	246
19335	"	1933	Leeds, N. Dak.	254	19347	"	1933	Morris, Minn.	253
19342	"	1933	Ft. Benton, Mont.	270	19281	Marquillo	1933	Minnesota	288
18738	"	1932	Scott, Sask., Canada	274	19287	"	1933	North Dakota	355
18723	"	1932	North Dakota	318	19288	Mindum	1933	North Dakota	472
18734	"	1932	Saskatchewan, Canada	322	19284	"	1933	Minnesota	513
19283	"	1933	Minnesota	330	19289	"	1933	Montana	690
19393	"	1933	South Dakota	335					

values than the hard spring wheats, though one Marquis and one Ceres sample were also very high.

Comparative Diastatic Activity of Flours Milled on a Long System Mill and on an Allis Experimental Mill

Seventeen samples of wheat were secured from the Northwestern Crop Improvement Association together with flours milled from them on the Pillsbury experimental mill at Minneapolis, Minnesota. Samples of these wheats were also milled on the Allis experimental mill in our laboratory. In Table VII are recorded

TABLE VII

COMPARISON OF DIASTATIC ACTIVITY OF FLOURS MILLED ON AN ALLIS EXPERIMENTAL MILL WITH FLOURS MILLED ON A LONG SYSTEM MILL. *Milligrams of maltose per 10 g. of wheat-meal or flour*

Serial number	Variety	Production area	Maltose		
			Wheat	Flour L	Flour S
			Mg.	*Mg.*	*Mg.*
19334	Marquis	North Dakota	210	226	166
19336	Reward	" "	211	272	144
19339	Reward	" "	216	252	162
19331	Marquis	"	218	230	130
19332	Ceres	" "	239	259	183
19338	Ceres	" "	242	330	170
19341	Marquis	Montana	244	232	130
19343	Thatcher 2303	"	244	230	128
19340	Thatcher 2303	North Dakota	246	270	152
19347	Thatcher 2303	Minnesota	253	—	168
19335	Ceres	North Dakota	254	290	204
19333	Reward	" "	256	256	166
19345	Montana 649	Montana	260	294	146
19337	Marquis	North Dakota	262	219	136
19342	Ceres	Montana	270	278	168
19344	Supreme	"	270	211	124
19346	Marquis	Minnesota	279	275	160
Average			246	258	155

the results of diastatic activity tests conducted on the two sets of flours, together with the corresponding results obtained on the wheats. The flours are distinguished by L (long) and S (short) milling systems, respectively.

The outstanding facts shown by the data in Table VII are the large differences between the flours milled on the two different mills. The short system flour values were only about 60% of the values found for the long milling system. These differences were also reflected in baking tests. When the long system flours were baked without sugar

the loaves had a much better volume and texture than flours from the same wheats milled on the experimental mill. The causes for these differences have not been studied.

Influence of Moisture Content

Wheat containing 15.5% moisture. A question of practical importance is whether the maltose content or the diastatic activity can be increased by adding moisture and storing, as is the case in long tempering before milling. To secure information at this point, 200 g. portions of wheat, containing 10% moisture, were placed in flasks and sufficient water added to raise the moisture content to 15.5%, after which the flasks were stoppered with cotton wads. They were then left in the laboratory for 4, 8, 16, 24, 48, and 96 hours and 1 and 2 weeks. At the end of each period the wheat was ground and the sugar content and diastatic activity determined. The sugar content (milligrams maltose soluble in water before digesting) and the diastatic activity (milligrams maltose formed during digestion for one hour at 30° C.) are given in Table VIII. The first column gives the milligrams maltose before digestion, or that which was present in the wheat, the second column the milligrams after digestion which shows the diastatic activity. Thus, the figures which follow "sugar content" mean that present in the wheat, while those for "diastatic activity" mean the total maltose obtained after one hour's digestion.

TABLE VIII

SUGAR CONTENT AND DIASTATIC ACTIVITY OF WHEAT CONTAINING 15.5% MOISTURE AFTER VARYING PERIODS. *Milligrams of maltose per 10 g. of wheat-meal*

Period	Sugar content	Diastatic activity
	Mg.	*Mg.*
Check	78	274
4 hours	90	230
8 "	82	230
16 "	98	222
24 "	116	251
48 "	114	258
96 "	102	242
1 week	104	235
2 weeks	106	218
2 " (55° F.)	120	210

Wetting wheat and letting it stand for varying periods did not increase the diastatic activity and the increase in sugar content was small. This shows that the amount of tempering water ordinarily used will not increase the diastatic activity. These data agree with findings previously reported (Swanson, 1934) in which wheat wetted to 12, 14,

16, 18, and 20% moisture and let stand for 16 weeks, showed a decrease in maltose content, except at 20% moisture and where mold growth occurred.

Affect of varying moisture content and keeping time uniform. In another experiment the moisture contents of portions of wheat were varied from 10 (check) to 42%. The same methods were employed as in the preceding experiment, except that the time was 72 hours for all samples. The flasks were weighed at the end of 24 and 48 hours and the moisture lost, about 1 cc., by evaporation was replaced. At the end of 72 hours the wheats were emptied from the flasks into shallow pans and exposed in the laboratory until air dry. They were then ground and the maltose present before and after one hour's digestion determined. The results are given in Table IX.

TABLE IX

MALTOSE PRODUCED IN WHEAT WETTED TO VARIOUS MOISTURE CONTENTS AND LET STAND FOR 72 HOURS. *Milligrams maltose per 10 g. of wheat-meal*

Moisture content	Sugar content	Diastatic activity
%	*Mg.*	*Mg.*
10	84	245
15	56	246
20	80	263
24	80	220
27	80	370
30	105	500
33	140	570
36	262	755
39	235	820
42	290	1060

It is clearly evident that there was no increase in the maltose present until the moisture content was raised to 30%, and no increase in diastatic activity until it reached 27%. Visible signs of germination were evident on samples with 36% moisture and above, and the process apparently existed in the incipient stages at a moisture content of 27%.

Effect of varying both the moisture content and time. In another experiment the moisture content of the wheat was varied from 15 to 42%, and the samples allowed to stand for 24, 48, and 72 hours. At the end of the indicated periods the wheat was air-dried, ground, and the maltose determination made. The results are given in Table X.

No increase in sugar occurred with any moisture content in 24 hours, only with 42% moisture in 48 hours; and with 36% and above moisture contents in 72 hours. Increase in diastatic activity took place at 33% moisture after 24 hours, and at 30% after 48 and 72 hours. Figures in Tables IX and X indicate that increase in sugar content or in diastatic

TABLE X

MALTOSE PRODUCED IN WHEAT WETTED TO VARIOUS PER CENTS AND ALLOWED TO STAND 24, 48, AND 72 HOURS. *Milligrams of maltose per 10 g. of wheat-meal*

Moisture content	Before digestion for			After digestion for		
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
%	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
15	46	38	42	385	235	260
20	44	42	46	269	234	225
25	40	38	42	264	246	234
30	42	38	38	296	365	433
33	42	46	44	396	525	590
36	46	38	50	390	535	695
39	38	42	72	433	563	750
42	38	52	80	458	615	790

activity does not occur until the amount of moisture is sufficient to start the process of germination.

Effect of alternate wetting and drying. It is generally thought that wet weather during harvest influences the diastatic activity. An attempt was made in the summer of 1933 to study the effect of artificial wetting. Wheat was wetted by sprinkling with water both while standing and after it was placed in the shock. Some bundles were left to dry in the sun and others were placed in a shed. These wheats were tested for diastatic activity, milled and baked, using low sugar in the formula, but no appreciable effects from wetting were observed. It should be mentioned that the weather was very hot and dry and hence the treatments were not comparable to cloudy and rainy weather. The results indicated, however, that a small rain followed by sunshine probably has very little effect. It is probable that insufficient moisture was absorbed by the wheat kernel to start the changes associated with germination. When changes in diastatic activity are effected during harvest they apparently result from considerable rain or rain in connection with cloudy weather.

In order to test further the effect of wetting, samples of selected plump hard winter wheat were soaked in water for 10, 20, and 30 minute periods, respectively, then dried by exposing to laboratory air 24 hours. This process of wetting and drying was repeated daily for 2, 3, and 4 days on other series of samples. After the last soaking the samples were exposed until completely dry, after which the sugar content and diastatic activity were determined. The results obtained are given in Table XI.

The sugar content was not appreciably increased by the 10 or 20 minutes exposure, nor by the 30 minute exposure until after 3 and 4 soakings. A decreased activity was noted in several instances probably due to the increased respiration consuming the sugar more rapidly than

TABLE XI

EFFECT OF REPEATED SOAKING AND DRYING GRAIN ON ITS SUGAR CONTENT AND DIASTATIC ACTIVITY. *Milligrams of maltose per 10 g. of wheat-meal*

Times soaked and dried	Soaked 10 minutes		Soaked 20 minutes		Soaked 30 minutes	
	Sugar content	Diastatic activity	Sugar content	Diastatic activity	Sugar content	Diastatic activity
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	78	240	76	213	60	315
2	78	248	90	315	55	247
3	78	340	80	405	95	390
4	78	420	90	425	93	470
Check	84	264				

it was formed. Increased diastasis was evident with 3 soakings for 10 minutes, two soakings for 20 minutes, and with one soaking for 30 minutes. The low value obtained with two soakings for 30 minutes was probably due to some condition not observed.

The experiment of alternately soaking and drying was repeated using two groups of samples. In one group the wheat was dried after soaking as in the preceding experiment, and in the other the grain was left in wide mouth bottles where it remained wet for a considerable time between soakings. After the last soaking all samples were thoroughly dried and the sugar content and diastatic activity determined as in previous experiments.

TABLE XII

MALTOSE CONTENT AND DIASTATIC ACTIVITY AS EFFECTED BY ALTERNATE WETTING AND DRYING. *Milligrams of maltose per 10 g. of wheat-meal*

Times wetted	Dried after each soaking				Not dired after each soaking			
	Soaked 10 minutes		Soaked 30 minutes		Soaked 10 minutes		Soaked 30 minutes	
	Sugar content	Diastatic activity	Sugar content	Diastatic activity	Sugar content	Diastatic activity	Sugar content	Diastatic activity
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	83	235	63	195	86	287	105	442[1]
2	84	245	74	228	87	315[1]	131	435[1]
4	62	315[1]	110	410[1]	82	407[1]	153	735[2]
Check	96	340						

[1] Small amount of germination.
[2] Large amount of germination.

There was no increase in either the maltose present or that produced by one hour of digestion in the samples dried immediately after each soaking, except when the 30-minute soaking was repeated four times. On the contrary, there was a decrease, probably due to increased respiration. All the samples soaked 30 minutes and not dried showed an increase both in sugar content and in diastatic activity. The samples soaked 10 minutes and not dried showed an increase in diastatic activity but not in sugar. In most of the samples showing an increase in sugar content or diastatic activity there was evidence that germination had started. Thus it appears that the process of germination is necessary for the increase in sugar or diastatic activity.

Development of Maltose in the Germ and Brush Ends Respectively

It is known that the special function of the epithelial layer of cells lying between the germ and the endosperm is to secrete enzymes, provided the conditions are such as to stimulate germination. To what extent enzymes can be stimulated or formed outside this epithelial layer does not seem to have been investigated. To gain information on this point wheat kernels were cut into two parts, germ ends and brush ends, and the two samples as well as the whole wheat kernels were kept in the germinator for 72 hours; after which they were dried, ground, and the maltose content and diastatic activity were determined. Two wheats, a hard red winter and a white, were used. The results of the experiment are given in Table XIII.

TABLE XIII

COMPARISON OF THE DIASTATIC ACTIVITY OF THE GERM AND BRUSH ENDS OF WHEAT KERNELS. *Milligrams of maltose per 10 g. of wheat-meal*

Sample	White wheat		Red wheat	
	Sugar content	Diastatic activity	Sugar content	Diastatic activity
	Mg.	*Mg.*	*Mg.*	*Mg.*
Check, *not germinated*	68	240	50	277
Whole wheat, *germinated*	415	1050	565	1750
Germ end, *germinated*	720	1800	775	1225
Brush end, *germinated*	130	750	340	850

While these data are limited they indicate that during germination the greatest formation of diastase occurred in the germ end, but considerable diastasis also occurred in the brush end. The presence of the enzymes in the brush end is known from the diastatic activity of flour which is practically germ free. Since about half the endosperm was present in the germ end, the enzyme content of this fraction was prob-

ably derived from two sources: (1) the enzymes of secretion or those derived from the epithelial layer between the germ and the endosperm, and (2) the enzymes left in the endosperm at the time of kernel building. On the other hand, the brush end probably contained only the latter group.

The stimulation of the enzyme activity during germination is thus dual, the secretion of enzymes by the epithelial layer and the activation of those already present probably as proenzymes.

Discussion

Two of the most important factors in dough fermentation are gas production and gas retention. The latter depends largely on the quality of the wheat proteins, the former on the condition for yeast growth. One of the major conditions affecting yeast growth is an adequate supply of sugar. The importance of diastatic activity is in connection with the supply of sugar. When enough sugar is added in the dough, diastatic activity becomes of minor importance. It becomes of increasing importance as the sugar content of the dough decreases or when the amounts of sugar are such that it is likely to be lacking during the proofing or the last stages of fermentation. If the yeast is starved during the proofing, poor bread will result regardless of the quality of the protein. If the flour is sufficiently high in diastatic enzymes, these will convert some of the starch to sugar and so furnish the yeast food.

The wheat kernel was not made to be milled into flour but to produce another plant. The starch, protein, and other substances were stored in the kernel to feed the embryo plant until it becomes large enough to take its nutriment from the soil and air. The foods stored as complex compounds in the wheat kernel must however be changed to simpler forms before they can be used to build the infant plant. During the process of kernel formation enzymes of various kinds are present in relatively large amounts. During the process of desiccation or ripening the kernel is modified so as to be able to preserve life under conditions which would be fatal to the growing plant. Accompanying the process of desiccation the enzymes become relatively inactive. The desiccation in hard wheat usually continues until the moisture content is from 10 to 13%. At this moisture content, respiration goes on in the kernel at a very slow rate. In other words the wheat is living in a state of dormancy.

That enzymes, at least the diatases, are distributed in the endosperm is known from the fact that they are found in the flour. That these respond to stimulation was shown by the experiment with the brush end of the kernels. These enzymes are probably in the form of proenzymes. The large increase in enzyme activity when conditions are favorable for

germination, is probably due to the enzymes of secretion. Between the embryo and the endosperm there is a layer of cells, the special function of which is to secrete enzymes, including, diastases, proteases, lipases, and others, when the proper stimuli are applied. The chief stimulus is moisture with a suitable temperature. Under this stimulus the secretion of enzymes starts and a large portion of these filter into the endosperm where they change the starch and protein into simpler compounds. The germ itself is particularly rich in protein and fat, and these are also changed into less complex compounds. To carry on this process there must be present enough moisture for the secretion, for the hydrolytic changes involved, and for diffusion. From the experiments reported, it seems that 27 to 30% moisture is enough to start secretion of enzymes, but not enough to carry on the subsequent processes. For this latter purpose about 40% moisture seems to be the optimum. This is about the amount which the kernel absorbs when in contact with free water. At such a moisture content the germination processes go on very rapidly if other conditions are favorable.

Moisture is thus the chief stimulant to increase the diastatic activity, but such amounts of moisture as may be used in wheat tempering (about 15.5%) are not effective. The amount of moisture must be large enough to start the process of germination. The temperature must also be suitable, as at too high a temperature the sugar is not increased nor does the germination take place as was shown by the experiments of Swanson, Fitz, and Dunton (1916). The effect of low temperature was not included in these studies, but wheat is known to germinate slowly at rather low temperatures. The effects upon diastatic activity of an amount of moisture such as would be absorbed from rains of short duration followed by drying weather seems to be nil.

To what extent inheritance or variety is a factor in diastatic activity is difficult to determine in the common wheats (*Triticum vulgare*). That durum wheats (*Triticum durum*) are comparatively high in diastatic activity has been shown in these experiments as well as by Mangels (1926) and others. The hard spring wheats seem to be higher in activity as a rule than the hard winter wheats, but the soft winter wheats were found to be relatively low. While variety is no doubt a factor influencing diastatic activity, it does not appear as dominant as some factors operating during ripening and harvesting, or artificial wetting. The latter factor is well understood in connection with the processes of malting barley. More information is needed concerning the factors which are operative during ripening and harvesting of wheat that influence diastatic activity. These studies are being continued.

Accompanying diastatic activity there is also protease activity. That the protease activity of flour is very slow was shown by Swanson and

Tague (1916). This is probably due to the fact that a relatively small amount of the proteases are found in the pure endosperm. Some of the benefits to baking derived from adding small amounts of flour from sprouted wheat seem to be out of proportion to the increased saccharogenic activity. At least, in many instances improvement from such addition results when there is no lack of sugar in the dough. This improvement is probably due to increased protease activity which helps to modify the gluten. It is hoped that this problem may be investigated further.

Summary

Diastatic activity varies in different classes of wheat. It was found to be highest in durum wheats followed by the spring wheats. Some white wheats were found to be comparatively high and others to be low. Soft red winter wheats as a class were lower than the hard winter wheats. Wheats grown under very dry conditions were lower in their activity than those grown under more moist conditions. The same variety of wheat grown in different localities showed a large variation, probably induced in response to environment.

The flour milled on an experimental Allis mill had a lower diastatic activity than the flour milled from the same wheat on a long system mill, the former was considerably lower than that of the wheat, while the latter was more nearly equal to that of the wheat.

Diastatic activity is not increased by wetting wheat until the moisture percentage in such wheat is sufficient to start the process of germination. Alternate wetting and drying is not effective in inducing changes in activity unless the wet condition is continued for considerable periods.

The diastatic enzymes in the endosperm, and consequently in the flour, probably exist in the form of proenzymes which may be activated by water. The amount of such enzymes present probably depends on conditions during kernel formation and ripening. Enzymes are also secreted by the epithelial layer of cells. These are increased under germinative conditions. For this reason flour from malted wheat is rich in enzymes.

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A STUDY OF THE PHYSICAL PROPERTIES AS WELL AS SOME OF THE CHEMICAL PROPERTIES OF DRIED EGG ALBUMEN WITH THE VIEW OF STANDARDIZING THIS SUBSTANCE FOR THE OFFICIAL CAKE BAKING TEST

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At the meeting of the American Association of Cereal Chemists held in 1933 it was decided that the Committee on Cake Baking Tests further study the materials used with the hope that they might be standardized so that the results obtained would be consistent in scientific practice. The materials subject to study are milk, shortening, the leavening agent, and egg albumen. This paper is concerned with a study of the dried egg albumen used in the baking test.

In a study of 180 samples of dried egg albumen over a period of time it was found that there were marked differences in the quantity of coagulated material, the quantity of dirt that came out of the dissolved albumen, the color of the albumen, the color of the foam produced, the specific gravity of the foam obtained when dissolved albumen is beaten, and the hydrogen-ion concentration of the albumen after it had been soaked in water. The specific gravity of the foam varied from 0.206 to 0.080.

A study of the data evolved the thought that the variation of these properties warranted a further study of the dried egg albumen to determine if the variation of the properties would be reflected in the cake baking test.

Up to recent years practically all of the dried egg albumen was prepared in China. The manufacturers of this product exercised very little control over its production and it was, therefore, subject to bacterial and enzyme changes. Blomberg (1932) in his discussion of the manufacture of dried albumen, states: "According to present practice the albumen must first be fermented because fresh dried albumen will not whip to a stable froth when reconstructed. The fermentation consists of holding the whites in large casks from 36 to 60 hours. A sediment is produced which is filtered from the albumen solution and the clear albumen is dried by placing it in shallow zinc or aluminum trays

and held at temperatures of from 120° to 140° F. for 40 to 45 hours." He also discusses the drying of egg albumen by placing it on a continuous belt and by the spray drying process.

The changes that may take place in the structure and composition of the albumen can occur during the so-called fermentation period and during the drying process.

Wu (1931), in a study of albumen, presents data in support of the hypothesis that the molecule of natural soluble protein is not a flexible open chain of polypeptides but has a compact structure. The force of attraction between the polar groups in a single molecule of protein holds them together in an orderly way just as the force of attraction between molecules holds many molecules together in a crystal. In coagulation the compact and orderly structure is disorganized.

Komatsu and Kotake (1931) have shown that the hydrolysis of egg albumen at 120° F. for 20 hours and at 150° F. for 9 hours indicates that the soluble part increased with the time of digestion, and that the amino nitrogen in the soluble part increased with the time of digestion at 120° F. and 150° F. while the diamino nitrogen showed a slight decrease. In comparison with the original protein the soluble part decreased in leucine, glutamic acid and tyrosine. In studying the optical properties of leucine and glutamic acid no change was observed in the leucine but a change was noticed in this property of the glutamic acid.

Schweitzer (1932), in his study of the physio-chemical process in the aging of egg albumen, made measurements of the pH value, viscosity, osmotic pressure and resistance to flocculation. He states that none of the results is uniformly consistent with what might be expected at the iso-electric point of a solution and the changes with time were so small as to be within the limits of error. The only conclusion he was able to draw was that if the pH value was less than 9.4 the egg may probably be fresh, while if the pH value is 9.4 or more the egg in all probability is at least eight days old. These data, at least, indicate that changes do take place in egg albumen.

Experimental

Series I

In our study of this substance we undertook the determination of the pH values; the measurement of the surface tension; the beating property; the determination of the specific gravity of the foam produced; the baking test of each albumen; a determination of the specific gravity of the cake batter; a penetration determination according to Hill (1928); surface tension measurement of the batter; pH value determination of each batter; the score of each cake according to the

A. A. C. C. system; photomicroscopic studies of the cell structure of each cake produced; determination of the swelling power of the crumb of cake containing egg albumen, and the same determination of cake made of a mixture in which no albumen was used.

In setting up our procedure we suspended 12 g. of albumen in 70 cc. of water and permitted it to soak until its quota of water had been imbibed.

Making the pH Determination

In making the pH determination the calomel cell was used. The determinations were made at a temperature of 25° C. The results obtained in making this determination in the egg albumen, batter and the finished cake are given in Table I.

TABLE I

pH Values of Egg Albumen, Batter, and Finished Cake

Sample	No. 4	No. 7	No. 1	Fresh	No. 8	No. 3
pH of albumen solution	6.4	8.85	6.50	8.10	6.30	6.70
pH of batter	6.0	6.15	6.22	6.10	5.90	6.25
pH of cake	8.4	8.75	8.50	8.65	8.30	8.55

Note how the range of pH value of the egg albumen goes from the alkaline to the acid side. Sample 7, with a pH value of 8.85, was a flaked albumen which, when dissolved, resembled the properties of fresh egg white. The sample with a pH of 8.10 was a fresh albumen. All the other samples were finely ground products and were on the acid side.

Surface Tension Measurements

Surface tension measurements were made with a Becker surface tension balance employing a wire loop of 13 mm. diameter. Results are given directly in grams in Table II.

TABLE II

Surface Tension Measurements of Egg-Albumen and Batters

Sample	No. 4	No. 7	No. 1	Fresh	No. 8	No. 3
	Gms.	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*
Surface tension of albumen solution	.4370	.4104	.4500	.4362	.4000	.4156
Surface tension of batter	.4000	.3911	.3811	.3864	.3735	.3800

Beating Property

Each solution of albumen was then beaten in a Hobart machine at a beating speed of 580 r.p.m. The results obtained are shown in Figure 1. Note the differences in the beating characteristics of each one of

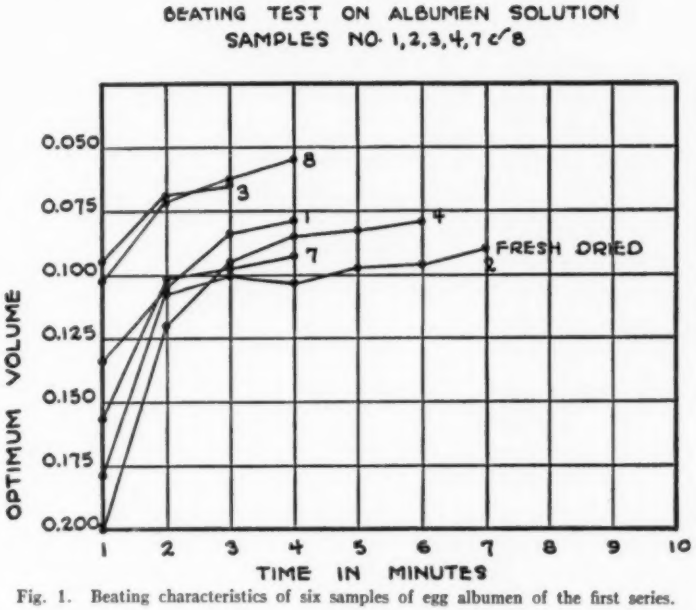

Fig. 1. Beating characteristics of six samples of egg albumen of the first series.

TABLE III

BAKING TESTS WITH ALBUMEN SAMPLES, FIRST SERIES

Sample number	1	2	3	4	5	6
Sugar, *grams*	250	250	250	250	250	250
Shortening, *grams*	65	65	65	65	65	65
Flour, *grams*	252	252	252	252	252	252
Milk, *cc.*	230	230	230	230	230	230
Egg whites, *grams*	82	82	82	82	82	82
Salt, *grams*	4	4	4	4	4	4
Soda, *grams*	3	3	3	3	3	3
Cream of tartar, *grams*	6	6	6	6	6	6
Baking time, *minutes*	35	36	34	35	33	34
Weight of cake, *grams*	293.7	293.5	294.3	290.7	292.7	293.6
Symmetry, *score*	6.0	8.5	5.0	9.0	7.5	6.5
Volume, *cc.*	7.5	12.5	7.5	12.5	10.0	10.0
Crust, *score*	3.0	4.0	2.5	4.5	3.5	3.5
Texture, *score*	17.5	25.0	17.5	20.0	22.5	17.5
Grain, *score*	12.5	22.5	17.5	17.5	20.0	15.0
Color, *score*	13.0	9.0	11.0	14.0	13.0	9.0
Total score	59.5	81.5	61.0	77.5	76.50	61.50
Penetration time of batter, *seconds*	29.6	21.8	18.0	21.8	20.0	15.0
Specific gravity of batter	0.881	0.873	0.851	0.878	0.872	0.865

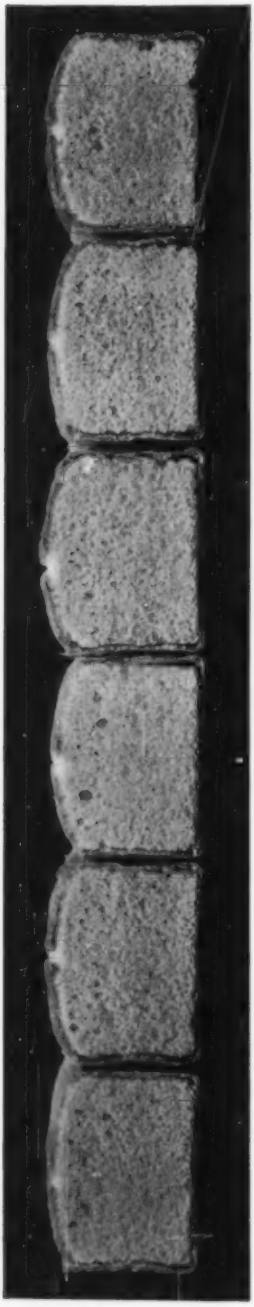

Fig. 2. Cross-section of cakes made with albumens of the first series.

these albumens. The fresh albumen, in this particular series, was beaten for a period of seven minutes before the optimum volume was reached. Sample 4 follows this same curve very closely up to six minutes. Samples 1 and 8 were beaten for a period of four minutes to reach their optimum volume, and sample 3 required only three minutes' beating time to reach its largest volume.

This indicates that there is not only a difference in the beating time required to reach the greatest volume in the samples in this series but that the optimum volume also varies, as is shown by the specific gravity obtained.

Baking Tests

In making baking tests the A. A. C. C. method of scoring was used. In Table III are recorded the scores which show marked differences in the various characteristics, also marked differences in the penetration test, with less variation in the specific gravity of the batter. Cross-sections of cakes made with albumens of the first series are shown in Figure 2.

Photomicrography of Cell Structure

Sections of the same area of each cake (see Fig. 3) were then taken and mounted. Each section was cut to 200 microns thickness. They were photographed at 25 magnification. The results obtained, according to the size of the voids, showed rather appreciable differences in the cellular structure of the cakes. This indicates that the albumen has an effect upon the character of the cell structure produced in the cake and that it varies with the characteristics of the albumen.

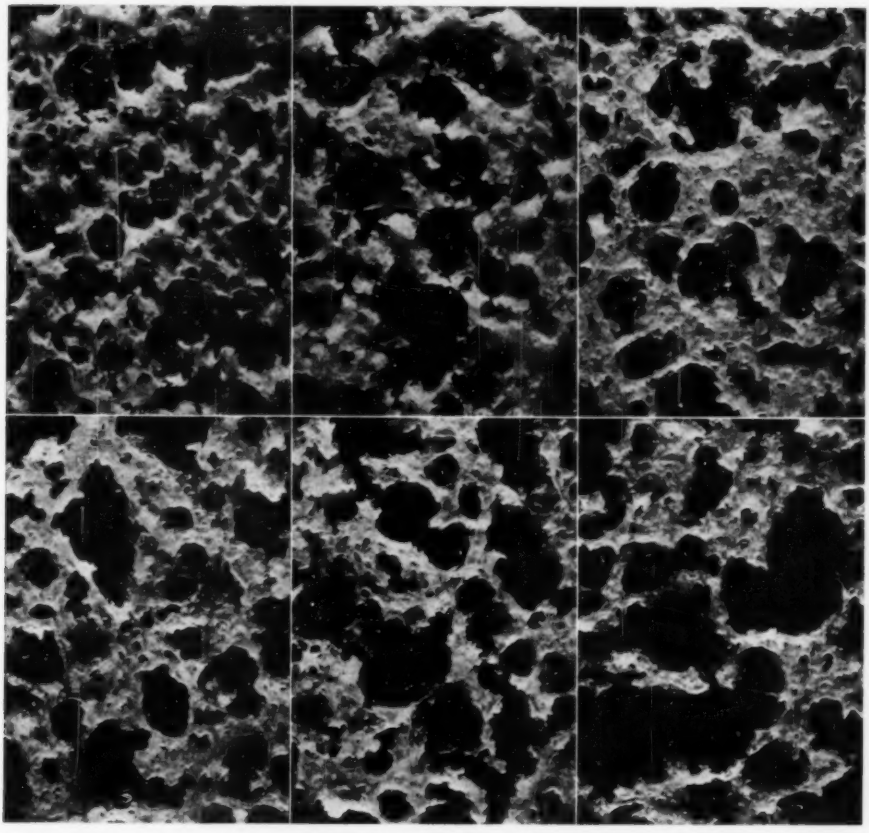

Fig. 3. Photomicrographs representing a cross-section of each cake made in which albumens of the first series were employed. Note increase of size of voids, reading from left to right.

Observations of Samples of Albumen Suspended in Water

In making up albumen solutions according to the A. A. C. C. method and placing these solutions into test tubes, the results shown in Figure 4 were obtained. The difference in the transparency and the settling out of solid material in three of the samples should be noted.

Swelling Power of Albumen

If cake containing an organized cellular structure is compressed to the point at which we obtain a ratio of 1 : 6 between the point to which it is compressed and the point of original volume and then suspend the discs in water, the mass will begin to swell and this swelling will continue until the cellular structure takes on its original volume. Water is imbibed very rapidly as the swelling process goes on. The rate at which the water is taken up varies with different cakes. It also varies

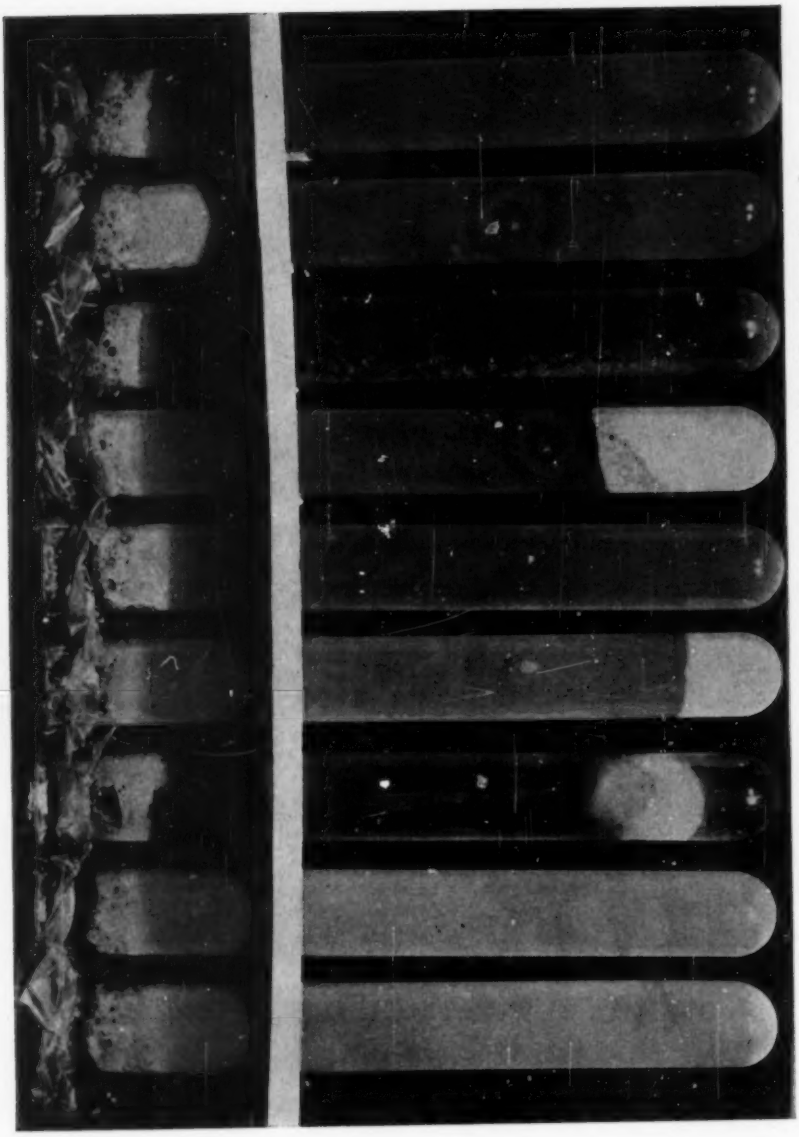

Fig. 4. A series of albumens in solution. The first series employed in this work was chosen from this number. Note the differences in transparency and the quantity of suspended material.

with the age of the cake. A point seems to be reached at which very little swelling takes place and at this juncture the mass disintegrates without going back to its cellular structure. In compressing 6 cm. of each sample of cake of the first series to 1 cm. thickness and then cutting a disc from each compressed mass, and suspending them in water, not only were differences in the rate of swelling obtained, but also an appreciable difference in the volume to which each disc rose under this condition was observed.

If a cake containing no albumen is compressed as described and then suspended in water we obtain a moderate degree of swelling and then disintegration takes place, beginning in the upper area. The difference between the swelling of the cake containing albumen and the cake containing no albumen is shown in Figure 5.

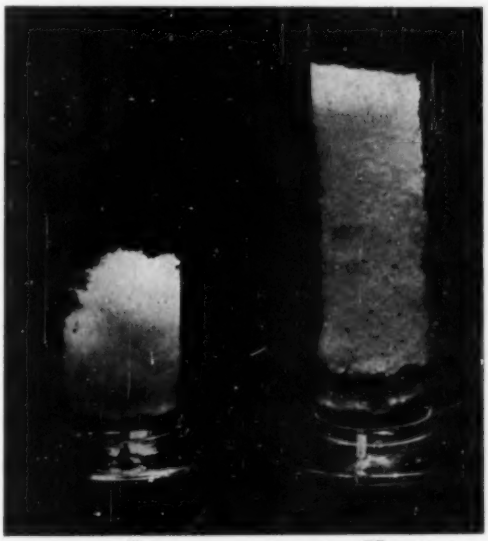

Fig. 5. Reconstruction of the cellular structure of cake.
No. I represents cake containing no albumen.
No. II represents cake containing a specified quantity of albumen as given in the A. A. C. C. method.

Observation of Surface Cleavage of Freshly Dried Egg Albumen

It was observed that egg albumen, in the process of drying, would take on definite surface characteristics when deposited upon slide glass and permitted to dry under controlled atmospheric conditions. Each surface would show certain lines of cleavage representing fissures of a definite pattern over the entire area. Photomicrographs were made of these surfaces, which are shown as Figure 6.

Fig. 6. Photomicrographic structure of a series of samples of dry albumen. Note the marked differences in the structure of the fissures.

Tests with Fermented Egg Albumen

The results obtained in the examination of samples of dried egg albumen prompted the investigation of liquid egg white when submitted to a fermentation process as employed in the preparation of dried egg albumen. In carrying on this work egg white of standard grade was selected. It was then placed into a sterile container and permitted

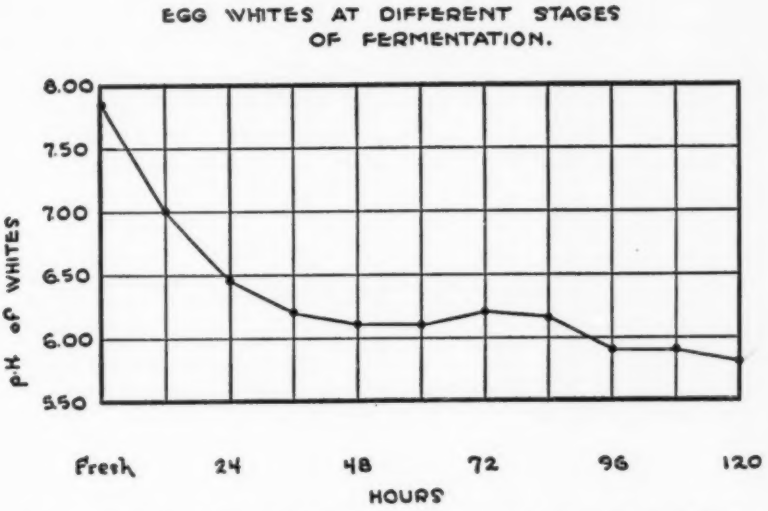

Fig. 7. Illustrating change that takes place in the pH value when fresh egg white is permitted to remain in an incubator for a period of 120 hours.

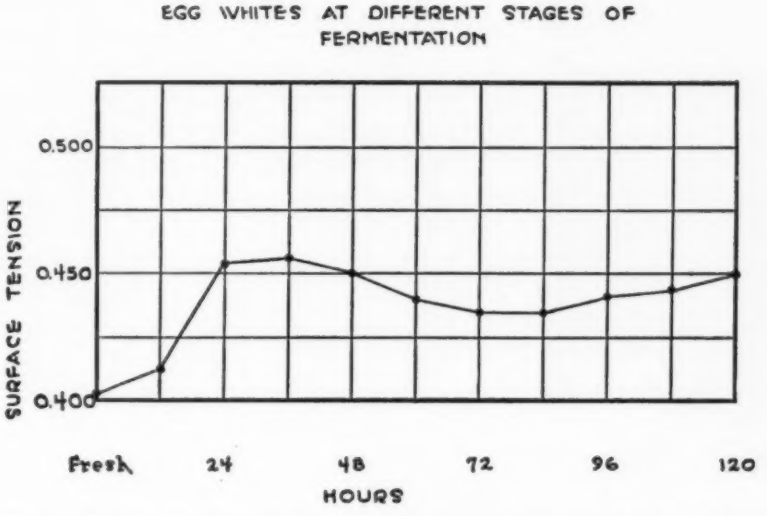

Fig. 8. Illustrating the change that occurs in the surface tension over a period of 120 hours.

Fig. 10. Cross-section of cakes made with albumens of the second series.

to remain under a constant temperature of 30° C. for a period of 120 hours. Samples were drawn at different intervals each day for the determination of the pH value, surface tension, beating property and the cake baking test.

Figure 7 records the pH value and Figure 8 the surface tension values for the liquid egg albumen over a period of 120 hours. The curve representing the pH value declines with the time while the curve representing the surface tension values goes upward with increase in acidity.

Series II

Beating Test

In making beating tests at daily intervals the results shown in Figure 9 were obtained. Note the differences in the beating characteristics of the albumen. The volume as represented in specific gravity does not go up as greatly in the fresh albumen but the beating time is extended over a period of nine minutes before the break occurs. In the other samples the volume rose to a greater height; that is, the specific gravity goes down to a lower degree, but the stability of the albumen is shortened considerably. These curves are more in keeping with those representative of the samples of the first series.

Baking Test

In carrying on the baking test the results given in Table IV and illustrated in Figure 10, were obtained. It will be seen that there is a variation in the score for the different characteristics, judged in accordance with the A. A. C. C. system.

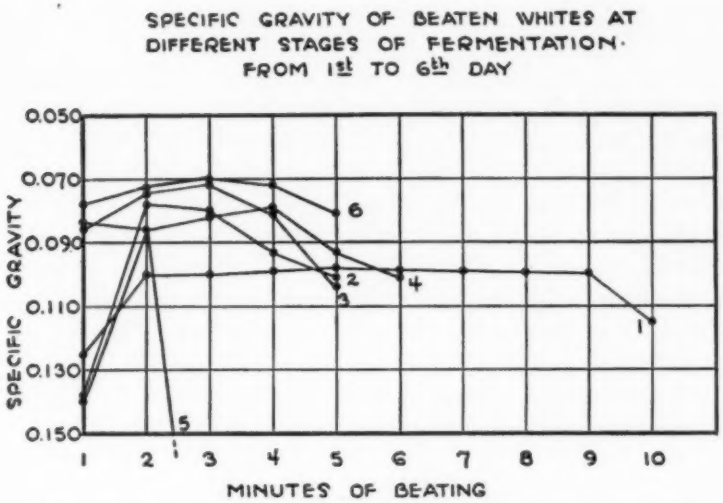

Fig. 9. Illustrating the beating property of fermented albumen when permitted to ferment over a period of 120 hours.

TABLE IV

BAKING TESTS WITH EGG WHITES AT DIFFERENT STAGES OF FERMENTATION

	Fresh	24 hours	48 hours	72 hours	96 hours	120 hours
Sugar, *grams*	250	250	250	250	250	250
Shortening, *grams*	65	65	65	65	65	65
Flour, *grams*	252	252	252	252	252	252
Milk, *cc.*	230	230	230	230	230	230
Egg whites, *grams*	82	82	82	82	82	82
Salt, *grams*	4	4	4	4	4	4
Soda, *grams*	3	3	3	3	3	3
Cream of tartar, *grams*	6	6	6	6	6	6
Baking time, *minutes*	34	36	35	34	36	32
Weight of batter, *grams*	325.0	325.0	325.0	325.0	325.0	325.0
Weight of cake, *grams*	292.7	291.0	291.1	295.5	294.4	292.3
Symmetry, *score*	9	5	9	8	9	7
Volume, *cc.*	11.25	5	12.5	8.75	10	7.5
Crust, *score*	4.5	3.0	2.5	4.5	4.5	3.0
Texture, *score*	27.5	17.5	22.5	20.0	20.0	17.5
Grain, *score*	23.75	15.0	20.0	17.5	15.0	12.5
Color, *score*	12.0	13.0	10.0	11.0	9.0	8.0
Total score	88.0	58.50	76.50	69.75	67.5	55.5

Summary

Our investigation of egg albumen, as found on the market, shows variations in the different characteristics studied. Some of these differences, at least, seem to be responsible for the variations in the structure

of the finished cake. These variations are apparent in all of the characteristics scored according to the A. A. C. C. scoring system.

Changes in the properties of the albumen affect the swelling power of cake that has been compressed to a definite degree of thickness.

The properties of the albumen seem to vary with the time the albumen is permitted to undergo the so-called fermentation process. Marked variations occur in the characteristics studied.

If a tentative standard is to be set up for albumen employed in the cake baking test, this standard should be based on the properties of domestic albumen, manufactured under known, controlled conditions. Samples having the same properties should be submitted to each member of the committee.

We would recommend further study so that more of the chemical and physical properties of albumen may be known.

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THE GRANULATION OF WHOLE WHEAT MEAL AND A METHOD OF EXPRESSING IT NUMERICALLY [1]

G. H. Cutler and G. A. Brinson [2]

(Received for publication October 27, 1934)

It is a matter of common knowledge that flours derived from different classes and types of wheat differ in respect to their granulation. Generally speaking, the hard wheats grind into coarse, rather granular flours, while the soft wheats give rise to fine, smooth, non-granular flours. Apparently, this is an inherent characteristic in these different classes of wheats. In recent years, a great deal of attention has been given to a study of flour granulation and its relationship to baking quality. Methods for studying this peculiar characteristic have been

[1] Contribution from Department of Agronomy, Purdue University Agricultural Experiment Station, Lafayette, Ind.
[2] Assistant Chief and Professor of Agronomy, and Graduate Student in Agronomy (1931–33), respectively.

given, but so far as the authors have been able to observe no attempt has been made to numerically designate the fineness of a given flour. Furthermore, there does not appear to be any published data concerning flour granulation as it relates to the wheat variety or strain, nor to the environmental conditions under which a given wheat is grown. These are problems of fundamental importance to the wheat breeder and farmer, since the miller chooses his wheats for milling with great care and in keeping with the requirements of the baker. Inasmuch as the miller of soft wheats and the baker of pastry products attach so much importance to flour granulation, considerable attention is being directed toward a study of this characteristic in the improvement and production of soft winter wheats in the Agronomy Department at Purdue University.

These investigations have been focused upon the granulation of whole wheat meal, since meal has been used in determining gluten quality by the fermentation time test (Cutler and Worzella, 1931 and 1933). This preliminary report will review these studies and describe the methods employed in expressing numerically the meal granulation.

The Granulation Test

The wheat sample: Dry, sound, clean wheat free from refuse and foreign material was used. Badly shrunken wheat was avoided. The wheat was kept under favorable storage conditions for several months prior to grinding, to allow the samples to become thoroughly air-dried to a uniform moisture content.

Grinding the raw wheat: Fifty-five grams of wheat were ground with a No. 1 Wiley Mill, equipped with a 1 mm. mesh sieve and set so as to grind a medium fine meal. Since the granulation of the ground meal will vary in keeping with the amount and nature of grinding, it is essential to observe a standardized technic in this important step in the granulation test. Meal ground for the fermentation time test of gluten quality is suitable for the granulation test. The Labconco Grinder has been adapted for this purpose and gives quite satisfactory results.

Fractionating the ground wheat meal: After the raw wheat is ground, it is fractionated by means of a mechanical device known as the Ro-Tap. This is an electrically driven machine which reproduces the circular and tapping motions of hand sieving with a uniform mechanical action. In our initial studies four metal sieves were used consisting of a 28 mesh, 60 mesh, 150 mesh, and a 270 mesh to the inch, and constituting a nest when fastened together. At the bottom of the nest is a pan for collecting the finest fraction of the meal particles. The sieves are eight inches in diameter and of standard height. In later experiments the method was simplified, so as to use only two metal sieves,—the 60 and

the 270 mesh to the inch. These appear to serve the purpose equally well and involve much less time in carrying out the test.

Ro-tapping procedure: The meal is "ro-tapped," if possible, the same day as it is ground. If this cannot be done, it is sealed in jars free from insects and vermin and where it cannot take on, or give off moisture. When ready to proceed, 50 g. of the meal are weighed out. Each sample is in turn placed on the top sieve and the Ro-Tap set in motion and allowed to run for one hour which permits of complete fractionation.[3] The meal on each sieve, as well as that in the pan, is then weighed and recorded and finally the granulation number is calculated.

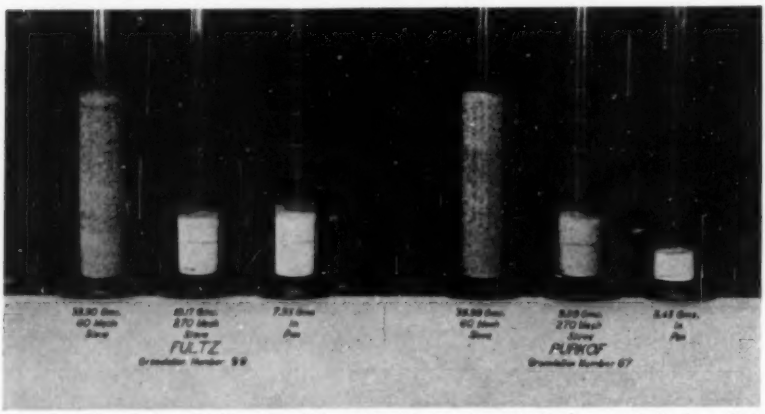

Fig. 1. The relative weights of meal in each of 3 fractions obtained respectively from Fultz, a soft variety, and Purkof a relatively hard variety, when 50 g. of wheat were used. These were grown at Lafayette in 1931–32.

Calculating the granulation of the meal: In attempting to arrive at a specific granulation number for each sample, the procedure takes into account all fractions including that which passes into the pan at the bottom of the nest of sieves. The authors have attempted to use all fractions on the grounds that the result is more accurate and representative, and furthermore lends itself to statistical studies.

In calculating the meal granulation number (see Table I) three assumptions are postulated as follows: (1) The size of the granules or particles of the meal for each fraction varies around a mean which is the same as the average size of the meshes of the two sieves (the one above and the one below) used in making each fraction, (2) the maximum size of the granules left on the top sieve is the same as the size of the mesh (1 mm.) through which all pass when ground with the Wiley Mill, and (3) the minimum size of the granules of the fraction

[3] A few kernels of wheat on the 270 mesh sieve prevents crusting of meal and aids in securing greater uniformity in sieving.

TABLE I

METHODS FOR CALCULATING THE GRANULATION NUMBER OF WHOLE WHEAT MEAL

Sieve sizes	Mean size of sieve mesh	Mean size of meal granule	Relative meal fineness[1]	Fraction[2]	Granulation number or index of fineness
	mm.	*mm.*		*g.*	*score*
1 mm. sieve (Wiley Mill) and 60 mesh Ro-Tap sieve	$\frac{1.0+.246}{2}$	.623	1.00	33.00	33.00
60 mesh sieve and 270 mesh sieve	$\frac{.246+.053}{2}$	.150	4.15	10.63	44.11
270 mesh sieve and average size of wheat starch granule	$\frac{.053+.022}{2}$	.038	16.40	6.37	104.47
				Total	181.58
					$181.58 \div 2 = 91$

[1] Linear rather than cubical measurement was used since it serves the same purpose and avoids large numbers.

[2] The data used represent the Michigan Amber variety of soft red winter wheat grown at Lafayette, Indiana, 1931–32.

which passes into the pan is the same as the average sized starch granule (0.022 mm.) as reported by Swanson (1928). On these bases four steps are required in calculating the granulation number of the whole wheat meal: (1) determine the mean size of the granule on each sieve (this is seen in column 3, Table I), (2) each of these results is then divided into .623 to ascertain the relative meal fineness of each fraction, (3) the relative meal fineness for each fraction is then multiplied by the amount of meal found on its corresponding sieve and results in its fineness or granulation number, and (4) the granulation number for the entire sample is then obtained by summing the numbers for each of the individual fractions. This number is usually quite large and for convenience sake is reduced by dividing it by two. It will be seen that when this technic is followed and the first two steps are completed they remain unchanged for other samples so that the third and fourth steps only are necessary in ascertaining the meal granulation number for each sample.

The granulation number thus obtained becomes the index or measure of the meal granulation of a given sample of wheat. The higher the number the finer is the granulation. The wheats preferred for pastry flours have a high meal granulation number while those preferred for bread flours have a low or relatively low granulation number.

Experimental

The meal granulation number of different varieties and classes of wheat grown at Lafayette: The first experiment consisted in testing the meal granulation of a large number of varieties of wheats representing the hard and soft winter classes. These were grown on the same general type of soil on the Soils and Crops Farm at Lafayette, in the seasons of 1931–32 and 1932–33, respectively. Table II records the data consisting of the meal granulation number, kernel texture (starchiness), and gluten quality.

Though the data represent but two years tests, they permit of interpretations that have an important bearing upon the problems in soft wheat improvement, production, and processing. These may be set forth as follows:

(1) The meal granulation of a large number of varieties studied representing the hard and soft classes varied from as low as 60 to as high as 120 and 130.[4]

(2) In general, it may be stated that the meal granulation numbers agree well with known facts concerning the flour granulation derived from these two classes of wheat.

(3) When subjected to the meal granulation test, these varieties can be grouped into three or more quite distinct groups.

(4) Within each group there are important varietal differences in meal granulation.

(5) Varieties with the lowest granulation number are characterized by kernels with hard, vitreous texture, with some that are mottled and starchy. Furthermore, in a general way as the granulation number increases, the percentage starchiness or mealiness increases and the gluten strength (as measured by the fermentation time test) becomes weaker. In other words, these characteristics seem to be roughly correlated, the granulation number being directly correlated with starchiness and negatively correlated with gluten quality. The macaroni or durum wheats are not included in this discussion since they grind coarser than the hardest bread wheats and at the same time possess a weak gluten.

(6) The miller, especially the soft wheat miller, who uses the fermentation time test of gluten quality in selecting and binning wheats (many in Indiana are now using it) will find the granulation test an additional useful guide in choosing desirable wheat for his milling operations.

(7) In a wheat breeding program where the hard wheats are being crossed on the soft wheats to combine a high degree of winter-hardiness in soft winter wheats, it will be seen that the meal granulation becomes an important selection factor.

[4] A limited number of tests on spring hard wheats grown in the spring wheat region reveal a granulation number similar to those (some were lower) of the hard winters.

TABLE II

THE MEAL GRANULATION NUMBER, TEXTURE (PERCENTAGE STARCHINESS), AND GLUTEN QUALITY (TIME IN MINUTES) OF VARIETIES OF SOFT AND HARD WINTER WHEATS GROWN AT LAFAYETTE, 1931–32 AND 1932–33[1]

Variety name	Classification	Meal granulation (2-year average)	Starchiness or mealiness (2-year average)	Gluten quality based on time (2-year average)
		Number	%	*Minutes*
	Group I[2]			
Prelude X Kanred	Hard Red Winter	60	14	152
Superhard Blackhull	" " "	61	8	85
Michikof	" " "	63	3	385
Ridit	Unknown	64	31	44
21-2-11	"	65	29	180
Minessa	Hard Red Winter	66	28	129
Tenmarq	" " "	66	70	238
Purkof	Semi-hard Red Winter	67	11	257
Newturk	Hard Red Winter	67	36	79
Ashkof	" " "	67	24	199
Iowin	" " "	68	51	182
Karmont	" " "	68	42	62
Mosida	" " "	68	43	196
Nebraska No. 50	" " "	69	42	200
Kawvale	Semi-hard Red Winter	70	77	143
Blackhull	Hard Red Winter	72	22	90
Minhardi	Semi-hard Red Winter	78	51	122
Early Blackhull	Hard Red Winter	78	55	81
Beloglina	" " "	84	47	122
Odessa	Semi-hard Red Winter	84	65	229
Mean		69	38	159
	Group II[3]			
Albit	White Winter	80	54	31
Red Wave	Soft Red Winter	81	70	48
Michigan Amber	" " "	83	66	67
Farmers Friend	" " "	84	69	35
Bald Rock	" " "	85	49	43
Michigan Wonder	" " "	86	90	51
Mediterranean No. 31	" " "	87	83	41
Bearded Winter King	" " "	87	64	37
Wis. Ped. No. 2	" " "	88	65	70
Illini Chief	" " "	89	70	38
Velvet Chaff	" " "	89	81	40
Fulcaster No. 84	" " "	90	90	40
Forward No. 92	" " "	91	80	46
Snow	" " "	91	89	—
Canadian Hybrid	" " "	91	82	36
Gipsy	" " "	91	91	39
Buffum's No. 17	Soft to Semi-hard	91	62	84
Bushel	Soft Red Winter	92	95	40
Fulcaster	" " "	93	89	41
Trumbull	" " "	93	60	39
Fulhio	" " "	93	61	39
Jones Fife	" " "	93	90	29
Forward No. 99	" " "	94	77	50
Currell	" " "	94	82	41
Nigger	" " "	94	91	35

TABLE II—(*Continued*)

Variety name	Classification	Meal granulation (2-year average)	Starchiness or mealiness (2-year average)	Gluten quality based on time (2-year average)
		Number	%	*Minutes*
Purdue No. 1	Soft Red Winter	94	73	35
Harvest Queen	" " "	95	94	46
Fultz	" " "	96	82	50
Goens	" " "	96	61	45
Acc. No. 128	" " "	96	87	46
White Fultz	" " "	96	90	39
Fultzo-Mediterranean	" " "	97	83	42
Nebraska No. 28	" " "	97	82	41
American Banner	White Winter	97	74	27
E. G. Clark No. 40	Unknown	97	45	45
Gladden	Soft Red Winter	98	75	40
Junior No. 6	White Winter	99	93	29
Mean		92	77	43
	Group III[4]			
Little Red	Soft Red Winter	100	91	39
Leaps Prolific	" " "	101	92	37
Kentucky Fultz	" " "	101	90	47
Roosevelt	" " "	101	92	45
Portage No. 122	" " "	102	96	40
Huron	" " "	102	85	27
A.P.I. No. 112	" " "	102	93	37
Nittany No. 117	" " "	103	89	47
Silver Sheaf	" " "	104	76	38
Acc. No. 127	" " "	104	86	43
Portage Acc. No. 116	" " "	105	96	37
Red Chaff	" " "	106	82	47
Nittany No. 123	" " "	106	92	37
Red Russian	" " "	109	79	28
Valprize	" " "	115	96	37
Mean		104	89	39

Summary

Group	Number of varieties	Classification	Granulation No.	Starchiness	Time
I	20	Hard Winter	69	38	159
II	37	Soft Winter	92	77	43
III	15	Soft Winter	104	89	39

[1] The moisture content of these samples was approximately 10%.
[2] The varieties in Group I were characterized in general by mottled and vitreous kernels.
[3] The varieties in Group II were somewhat mottled in texture but the kernels were predominantly starchy or mealy.
[4] The varieties in Group III showed little or no mottling, the kernels were almost wholly starchy or mealy in texture.

The Meal Granulation of Wheat Varieties When Grown Under Different Conditions

When eleven varieties of winter wheats, some of which were hard and some soft, were grown for two seasons at different outlying stations in Indiana where the conditions of soil and climate vary rather widely; it was quite surprising to note how little the meal granulation varied. From station to station and from season to season the granulation number of each variety showed little tendency to vary.

This is well illustrated in Table III where the granulation numbers of five varieties are shown for the seasons 1931–32 and 1932–33.

TABLE III

THE MEAL GRANULATION NUMBERS OF FIVE VARIETIES OF WINTER WHEATS GROWN AT SEVEN POINTS IN INDIANA IN EACH OF TWO SEASONS, 1931–32 and 1932–33

Area of production	Granulation number									
	Purkof		21-2-11		Bald Rock		Mich. Amber		Purdue No. 1	
	1932	1933	1932	1933	1932	1933	1932	1933	1932	1933
Vincennes	70	68	71	69	90	81	92	82	105	102
Bedford	70	77	69	71	88	84	90	92	111	118
Jennings Co.	71	67	66	67	83	81	96	87	112	104
Davis Farm	72	70	69	66	—	75	87	78	124	91
Huntington	71	75	71	71	—	86	92	95	117	114
Pinney Purdue (Wanatah)	70	75	70	70	79	88	98	89	128	106
Lafayette	67	66	68	62	99	71	91	75	102	86
Mean	70	71	69	68	88	81	92	85	114	103

An examination of the data indicates quite pointedly that the granulation of the meal from the whole wheat is a highly stable varietal characteristic.

Further confirmatory evidence of this fact is shown in the behavior of three other varieties of wheats when grown in three different states under widely different conditions (see Table IV).

Though the data are quite inadequate, they suggest certain trends which further support the general principle that the granulation structure of wheats is a relativley stable kernel characteristic. Further studies, however, will be necessary before definite conclusions can be drawn.

If the granulation of the wheat meal proves to be as stable as these data seem to suggest, the meal granulation test will prove especially help-

TABLE IV

THE MEAL GRANULATION OF WHEAT VARIETIES GROWN IN KANSAS, NEBRASKA, AND INDIANA

Varieties	Source	Meal granulation number—crop years		
		1931–32	1932–33	1933–34
Tenmarq	Kansas	—	64	—
Kanred	"	—	65	—
Blackhull	"	—	85	—
Tenmarq	Nebraska	—	64	62
Kanred	"	—	62	64
Blackhull	"	—	78	78
Tenmarq	Indiana	72	63	—
Kanred	"	—	—	—
Blackhull	"	77	70	—

ful in the identification of the low protein hard, yellow hard, mixed, or "bastard" wheats as grown under humid climatic conditions. The miller of pastry flour frequently experiences much difficulty in buying such wheats. He is unfamiliar with varietal peculiarities and not infrequently finds such characteristics as texture, protein content, and gluten quality rather misleading not to say undependable. Under such circumstances the meal granulation test may assume an important role.

The granulation test may also serve a useful purpose in the classification of wheats and the identification of wheat varieties.

Granulation tests of miller's samples: A limited number of samples of wheat supplied by Indiana millers have been tested for their meal granulation during the past two years. The results show that the wheats used by the soft wheat millers range from 90 to 110 in granulation numbers. Those wheats giving meals ranging from 100 to 110 were apparently the most popular for cake flours. When these data are correlated with the meal granulation of our wheat varieties representing the two main classes of common wheat (see Table III) they supply important fundamental information to the breeder and enable him to better understand the requirements of the miller and baker.

Summary

A test for measuring the granulation of whole wheat meal is described, and a method for expressing it as a specific number is illustrated.

A series of experiments and data have been presented indicating that the granulation of the meal varies widely among different classes and varieties of wheat.

Evidence is presented which suggests that the granulation of the wheat meal is a highly stable varietal characteristic. In this regard it seems possible that it may prove much more stable than kernel texture and gluten quality as measured by the fermentation time tests. In a general way there appears to be a positive correlation between the granulation of the meal and the starchiness or mealiness of the wheat kernel, and a negative correlation with the gluten quality as measured by the fermentation time test. The wheats preferred for bread flour have a low granulation number, while the wheats preferred for pastry flours have a high granulation number.

The meal granulation test is adapted for use of the wheat breeder but seems capable of being used also by the wheat buyer who is interested in purchasing wheats for specific purposes. It will serve as a dependable aid in identifying the low protein hard or yellow hard wheats that though relatively low in protein are too granular for manufacture into satisfactory pastry flours. It may also possess some value in classifying and identifying wheats.

The meal granulation test as described has been adopted as a method for ascertaining the desired granulation of new hybrids and selections in the soft wheat breeding program in the Agronomy Department at Purdue University.

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AN AUTOMATIC TIMING DEVICE FOR USE WITH A HOBART-SWANSON MIXER[1]

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Comparatively short mixing is required when a Hobart-Swanson mixer is used for routine baking tests and consequently it is highly desirable that the mixing should be accurately timed. A simple device to accomplish this was constructed in this laboratory and has operated satisfactorily for some months.

Operation

The device which is illustrated in Figure 1 is mounted on a plate R which is fastened to the mixer, behind the mixing head, by means of the bolts which hold the gear box on the bowl frame. The rotating spindle of the Hobart mixer was removed, leaving the other spindle A to actuate the device. When the mixer is in operation this spindle hits the arm B which is hinged at C, and carries it forward for about 1¼ inches. The arm then slips past the spindle and is pulled back to its original position against the stop T by the spring S. When the arm moves forward it pushes the plunger D through the bearing E. The motion of the plunger is transmitted to a ratchet G by means of the pawl F which is mounted on the end of the plunger. The spring S returns the plunger and arm assembly to their original position and the ratchet wheel is prevented from turning in the reverse direction by the pawl H. The ratchet actuates a pair of gears J and K which transmit the motion to a bakelite cam M. This cam raises the spring contact arm N until it makes contact at Q and the circuit remains closed until the cam has made one complete revolution. The contact arm then drops back to its original position opening the circuit. Since the circuit is open when the device is in the starting position, provision had to be made for starting the mixer. This is done by wiring the device to the motor through the switch so that the timer is shunted out when the switch is closed. In operation the switch is closed while the spindle A makes about four revolutions. By this time contact has been made in the timer and the switch is opened so that the circuit will be broken when the cam completes its revolution.

[1] Published as Paper No. 61 of the Associate Committee on Grain Research, National Research Council of Canada.

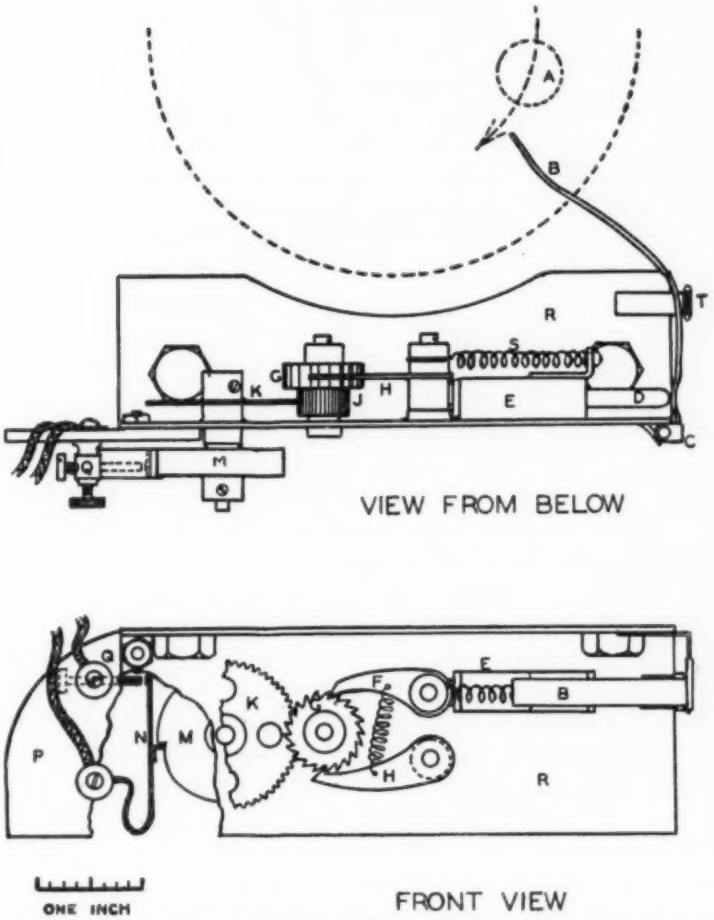

Fig. 1. Diagram of automatic timing switch for use with a Hobart-Swanson mixer.

If the mixer is run idle it will coast for about one revolution after the current is cut off. However, when there is dough in the bowl, the mixer stops as soon as the contact is broken.

Construction

A $\frac{1}{16}$ inch brass plate was bent at right angles, giving a base for mounting the mechanism $5\frac{3}{8} \times 2$ inches and a plate $5\frac{3}{8}$ inches $\times$ 1 inch for attachment to the frame of the mixer. Part of the edge of this plate was filed away to allow clearance for the rotating head of the mixer. The arm *B* was also made of $\frac{1}{16}$ inch brass. It was cut to a length of about $4\frac{1}{2}$ inches and the final length and shape were adjusted by trial. The hinge *C* was formed by soldering a brass yoke on this arm and by bending back part of the plate *R* about $\frac{1}{2} \times \frac{1}{4}$ inch to which

was soldered a small brass block. The block and the yoke were drilled to take a pin made from a small nail. The stop *T* was soldered to the main plate and the upright part covered with a piece of rubber tubing to act as a shock absorber. The plunger *D* was made from a piece of $\frac{3}{8}$ inch brass rod 3 inches long. This was machined to $\frac{3}{16}$ inch diameter for 1 inch from the end. This part was then turned up at right angles to provide a bearing for the pawl *F* and a collar was soldered on to keep the pawl from sliding down on the pin. The remaining 2 inches of the rod was filed to a square cross-section to fit the square guide *E*. A collar was soldered on to the end of the square section to prevent the plunger being pulled too far into the guide by the spring *S*.

The pawl *F* is a Meccano part (No. 147). The pawl *H* was cut from 16-gauge sheet metal and fitted with a brass collar. This pawl and the ratchet and pinion were mounted on $\frac{3}{16}$ inch pins soldered to the main plate. The ratchet *G* is $\frac{3}{4}$ inch in diameter and has 21 teeth. This is the only part which was specially made in an outside shop. The gears *J* and *K* are Meccano gears (26 and 27a) with 19 and 57 teeth respectively. The large gear is connected to the cam *M* by a $\frac{3}{16}$ inch shaft running in a collar-bearing soldered on to the main plate. The cam was cut from $\frac{1}{4}$ inch bakelite. This was mounted on a collar by filing down $\frac{3}{8}$ inch brass tubing to a square cross section and driving into a square hole cut in the cam. In order to avoid difficulties with insulation the terminals were mounted on a piece of $\frac{1}{8}$ inch bakelite *P* bolted to the main plate. The contact arm *N* was made from spring steel and a small arm was soldered on to this to run on the cam.

Modifications

The device, in the form just described, is adjusted to give mixing for one minute. If any other fixed mixing time is desired this may be obtained by using a ratchet with a different number of teeth or by altering the gear ratio between *J* and *K*. If it is desired to adapt the device so that it will give any one of a number of mixing times, this can be done quite simply by fitting a metal ring carrying a number of removeable studs, to the revolving head of the Hobart mixer. Removal of the studs will extend the time of mixing by reducing the number of times that the arm *B* will trip in each revolution of the mixer, and with a suitable choice of ratchet and gear ratio any desired range may be obtained. Use might also be made of the gear shift incorporated in the Hobart mixer but unfortunately there are not integral ratios between the high, medium, and low speeds.

It is possible also to use this device to control mixing time with the ordinary Hobart mixer.

CATALASE ACTIVITY IN WHEAT FLOUR[1]

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Subsequent to Loew's identification (1901) of catalase as an enzyme of general occurrence in biological material, several investigators have undertaken experimental studies pertaining to catalase activity in wheat products. Among those who have reported on this subject are Wender and Lewin (1904), Wender (1905), Liechti (1909), Bailey (1917), Marion (1920), Lindet (1920), Merl and Daimer (1921), Fernandez and Pizarroso (1921), and Bornand (1921).

These studies have invariably shown that in any sound, mature wheat kernel catalase activity is highest in the branny outer portion. It decreases progressively and very conspicuously toward the inner portion of the grain, and is lowest in the central part of the endosperm. Its distribution in the wheat kernel parallels that of the mineral content, or ash, of the wheat.

Parallelism with ash content has led several who have studied the situation to suggest the utility of catalase activity determinations for the purpose of indicating flour grade, or degree of refinement in milling. Bailey (1917) has noted the ease, convenience, speed, and simplicity of equipment with which catalase activity can be measured, although the degree of precision is possibly lower than that at which ash determinations can be made. He furthermore states, "in general we find that with double the ash content the catalase activity is increased to about 340% of the lower value, while treble the ash content accompanies a catalase activity of about 500% of the lower rate. The catalase activity thus increases at a more rapid rate than the percentage of ash. This is of distinct advantage in distinguishing between the various grades of flour, and compensates in large part for the diminished accuracy with which such tests can be made."

Considered in the aggregate, the results of previous studies do not furnish an adequate basis for judgment as to the *general* reliability of the catalase test as an index to flour grade. Different workers have used different methods, and the numerical values reported by one are therefore not strictly comparable with those of another. The writers

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have been unable to find instances where the same method was applied to flours from wheats of widely diversified type and origin.

Purpose of the Investigation

Having available an assortment of commercial bakers flours assembled (for another purpose) from widely scattered localities in North America, it was considered worth while to estimate their comparative catalase activities in an effort to obtain conclusive evidence as to the potentialities of the catalase tests as an indicator of the grade of flour. The individual ash contents of these flours, with but few exceptions, showed that they were of approximately equal grade.

Method for Estimation of Catalase Activity

All methods commonly used for estimating the activity of the enzyme catalase are based upon its distinctive and characteristic property of decomposing hydrogen peroxide with the liberation of molecular oxygen. One may measure the liberated oxygen by volumetric or manometric methods, or by estimating the amount of undecomposed hydrogen peroxide at a prescribed time interval. For comparable results, obviously, all measurements must be made under specified, uniform, and strictly controlled conditions of time, temperature, pH, etc.

Manometric methods are simple and convenient. They have been used in various forms by Liechti (1909), Appleman (1910), Bunzell and Forbes (1930), and others.

After some preliminary trials and modifications of procedure, the following basic method was found to give satisfactory and reproducible values:

A 2 g. sample of flour is weighed into a 125 cc. Erlenmeyer flask. This is thoroughly mixed with a teaspoonful of ignited sea sand, by vigorously rotating the flask. Six drops of oleic acid (foam preventative) are added; the flask is weighted with a lead collar, and almost completely submerged in a water bath maintained at 30° C. Another flask, containing a freshly prepared buffer-hydrogen peroxide mixture is also brought to 30° C. in the water bath. The buffer solution is a phosphate buffer of pH 6.8, and the buffer-hydrogen peroxide mixture is prepared so that 25 cc. of the mixture contains 24.5 cc. of buffer and 0.5 cc. of 30% hydrogen peroxide. When both flasks and their ingredients have reached the prescribed temperature, 25 cc. of the buffer-hydrogen peroxide mixture are added to the flour sample, and an S-shaped glass mercury manometer fitted into a rubber stopper of suitable size is forced as tightly as possible into the Erlenmeyer flask after first wetting the stopper with water. The weighted flask with manometer is then shaken by rotation for 30 seconds (under water) after which the upper mercury level is marked by a strip of gummed paper. At the expiration of exactly 15 minutes, the increased height of the mercury column is measured with a millimeter scale. This reading doubled gives millimeters of pressure produced by the oxygen liberated during the 15-minute time interval. This is recorded as the numerical value representing the catalase activity of the sample of flour used.

For purposes of these studies no attempt was made to convert results into absolute values or units; only *comparative* values were sought.

All materials and reagents used in the method were tested individually with hydrogen peroxide to insure that no ingredient other than the flour itself was a factor capable of causing any decomposition of the hydrogen peroxide. Values obtained by this method were found to be readily and consistently reproducible within 3 or 4 millimeters of pressure, whether replicate determinations were made on the same day or on different days.

Experimental Results with Variety of Bakers Flours

Using the procedure just described, catalase activity tests were made on 33 commercial bakers flours of approximately equal grade (with 1 or 2 exceptions) but widely diversified as to origin and also doubtless as to types of wheat from which they were milled. This miscellaneous assortment included samples from northwestern, midwestern, southwestern, Pacific Coast, and Canadian mills. All samples were two years old, but had been kept in cold storage at nearly freezing temperature.

Table I shows the catalase values obtained, and also presents data showing the source, the ash content, the protein content and the diastatic activity of each flour.

The data in Table I lead one very definitely to the conclusion that catalase activity parallels flour grade,—as indicated by ash content,—only when dealing with flours from wheats produced in the same general locality. Northern flours in general show far greater catalase activities than southwestern flours of equal grade. The Canadian flours give the highest catalase values while the Texas and Kansas flours run lowest. The latter average close to 40, or less, while the former average 137, or 3.5 times as much. Intermediate regions tend to show correspondingly intermediate catalase activity values.

Only one flour in the entire series appears to be very decidedly out of line with respect to the observed relationship between catalase activity and locality from which the flour was received. This is flour No. 8, from a Minneapolis mill, but with a very low catalase value. There is, obviously, no positive assurance that each of the flours was milled from wheat grown in the same locality as that of the mill which produced the flour. Thus flour No. 8 may have been made largely from a southwestern wheat even though milled in Minneapolis. It is, however, a reasonable supposition that most of the flours were, respectively, from wheats grown not far from the general region of the mills from which the flour samples were received.

The data in Table I offer no direct evidence as to whether or not wheat type or variety are important factors affecting catalase activity, because the types and varieties of the wheats involved were not known.

TABLE I

COMPARATIVE DATA ON MISCELLANEOUS BAKERS FLOURS

Flour No.	Location of mill	Catalase activity	Ash	Protein	Diastatic activity
		mm. pressure	%	%	*Rumsey units*
2	Lincoln, Nebr.	45	0.43	10.9	365
5	Kansas City, Mo.	52	.47	12.3	260
6	Minneapolis, Minn.	118	.47	15.1	160
7	Seattle, Wash.	83	.42	14.4	218
8	Minneapolis, Minn.	34	.41	11.9	227
9	Moosejaw, Canada	137	.45	13.3	170
10	Winnipeg, Canada	137	.48	13.9	192
11	Lincoln, Nebr.	97[1]	.62[1]	12.2	180
12	Wichita, Kans.	32	.41	12.1	164
13	Ft. Worth, Texas	43	.44	12.2	183
14	Minneapolis, Minn.	110	.46	13.7	173
15	Grand Forks, N. D.	76	.40	12.5	246
16	Denver, Colo.	50	.45	12.5	186
17	San Francisco, Calif.	71	.42	11.7	142
18	Toledo, Ohio	44	.45	12.2	215
19	Minneapolis, Minn.	80	.43	13.8	240
20	Kansas City, Mo.	36	.46	12.0	205
21	Wichita, Kans.	34	.41	11.5	212
22	Minneapolis, Minn.	66	.47	11.7	227
23	Galveston, Texas	34	.41	11.5	220
24	Seymour, Ind.	59	.52	10.7	233
25	Minneapolis, Minn.	61	.43	12.3	240
26	Danville, Ill.	39	.43	11.3	177
27	Kansas City, Mo.	40	.40	11.9	170
28	Topeka, Kans.	45	.44	11.7	184
29	Lexington, Nebr.	45	.48	11.1	233
30	New Ulm, Minn.	54	.47	11.8	213
31	Buffalo, N. Y.	86	.45	12.3	224
32	Chicago, Ill.	58	.42	12.4	228
33	Omaha, Nebr.	34	.45	11.3	252
34	Grand Island, Nebr.	41	.44	11.7	192
35	Hays, Kans.	40	.47	11.6	194
36	Buffalo, N. Y.	72	.45	12.2	260

[1] Clear.

In modern wheat production, however, the selection of varieties is itself very largely a regional consideration. One would reasonably expect, therefore, a high correlation between variety and catalase activity, assuming a given variety to have been produced in the region to which it is best adapted.

Further information on these matters was, however, obtained by testing five different varieties grown under identical conditions in the Agronomy field plots at the Nebraska Agricultural Experiment Station, and all experimentally milled to the same degree of refinement (85% patent). Two of the samples were spring, and three were winter varieties. The comparative catalase activity values of the flours from these wheats are shown in Table II.

TABLE II

WHEAT VARIETY AND TYPE AS FACTORS AFFECTING CATALASE ACTIVITY

Variety	Catalase activity
	mm. of pressure
Kanred	32
Kharkof	35
Blackhull	33
Marquis	66
Ceres	78

The data in Table II, although somewhat meager, indicate that spring wheat flours contain considerably more catalase than winter wheat flours, even though all wheats were grown under identical conditions. Wheat type or habit is apparently an important factor, while variety is not of importance among wheats of the same type or habit. Thus the three winter wheats gave closely agreeing values regardless of variety, while the two spring wheats gave comparable, though much higher, values.

The evidence offered by the data given in Table I is in general agreement with the findings of some other workers who have reported correlations between enzyme activity and climatic factors. Lischkewitsch (1930) found greater enzyme activity in wheat and barley grown in northern than in southern latitudes. Prokopenko (1927) reported lower catalase and peroxidase activity in winter wheat than in spring wheat. A Japanese Government report (De Sacy, 1927) states that rice grown in the cooler climates has higher catalase activity than that produced under warmer conditions. Ivanov (1932) reported relatively high catalase and amylase activities in barley and wheats grown in northern or mountain regions.

Table I gives the diastatic values of the flours under consideration. Apparently the systematic relationship found between catalase activity and region of wheat production does not hold for diastatic activity.

Is Flour Catalase a Single or a Multiple Factor?

Following the "catalase activity survey" of the series of miscellaneous flours, reported in Table I, some additional studies were undertaken for the purpose of gaining further insight into the nature of flour catalase activity. Under the conditions of the method hereinbefore described, is the hydrogen peroxide decomposing power of flour due to a single factor, present in all flours, but varying only in amount, or is it the resultant of two or more factors, occurring in varying ratios among different flours?

The latter situation was considered as a possibility in view of the fact that Loew (1901), and others have distinguished between two

forms of catalase, an insoluble "alpha" form and a soluble "beta" form. Furthermore, Balls and Hale (1932) state that under certain conditions peroxidase is capable of decomposing hydrogen peroxide, but that this factor may be eliminated if determinations are made at ice-water temperature. They also report that catalase may occur naturally in both active and inactive forms. They use liver extract for the purpose of activating inactive catalase, and dextrose as a protective agent against destruction of catalase by excess hydrogen peroxide.

In order to test the possibility that more than one factor might have influenced the catalytic decomposition of hydrogen peroxide, under the conditions of the manometric procedure previously described, a few typical flours showing high, medium, and low catalase values, respectively, were studied as to their action on hydrogen peroxide under different environmental conditions and after different treatments, all of the flours being treated alike in each set of conditions. If by some such means it were found possible to change the *order* or *degree* of variation among the flours in question, it might well be concluded that more than one factor is operative, on the assumption that two different biological agents cannot ordinarily be expected to respond in the same manner, and to the same degree, to the same change in environment.

Thus, for example, if a specified alteration in hydrogen-ion concentration is found to double the rate of reaction for one flour, but increases it only 25% for another, it may reasonably be suspected that at least two factors are involved, and that these two factors do not have the same optimum hydrogen-ion concentration. Conversely, if all flours respond in equal degree to the change in pH, there is presumptive evidence that a single factor is being dealt with.

Applying a number of "differential" treatments to a small group of flours having various catalase values, the following factors were included among those studied: (1) pH variations, (2) temperature of zero degrees (to eliminate peroxidase effects), with additions of dextrose and liver extract to give maximum activation with protection against catalase destruction by the hydrogen peroxide, (3) preliminary heat treatment of the dry flour, (4) addition of strong oxidizing agent, and (5) addition of strong reducing agent. Some typical results of these treatments, respectively, are summarized in Table III, following:

The data in Table III reveal a distinct tendency for the comparative values to remain in essentially the same order with respect to each other regardless of the treatment imposed. The *degree* of reduction in activity resulting from a given treatment is not always the same for the different flours. It is essentially so among the flours of higher catalase activity, but substantially lower in flour Px, whose activity by the basic procedure is however only 33. The trend of the results is sufficient

TABLE III

EFFECT OF VARIOUS TREATMENTS ON COMPARATIVE CATALASE VALUES

Flour No.	Catalase activity values (*in mm. of pressure*)						
	By prescribed method	At 0° with dextrose and liver extract	At pH 9.4	At pH 5.5	After heating dry flour for 1 hr. at 80° C.	Plus 0.05 g. of KIO_3	Plus 0.05 g. of Na_2SO_3
6	118	48	42	24	—	69	—
9	137	66	48	36	44	85	—
11	97	58	42	—	—	—	—
Px	33	28	25	13	16	24	24
Cx	105	50	33	32	37	57	71

to suggest strongly that for all practical purposes catalase activity in flour may be regarded as essentially a *single* rather than a multiple factor. If there are indeed two or more factors involved, these experiments offer no suggestion of any means whereby they might be distinguished from each other in a manner useful for purposes of identifying flour grade.

Solubility of Flour Catalase

In view of Loew's (1901) statement that catalase exists both in a soluble (beta) and an insoluble (alpha) form, a brief study of the distribution of these two forms in wheat flour was made. Some flours were extracted for one hour at 30° C. with the phosphate buffer at pH 6.8, and 25 cc. aliquots of the extracts were withdrawn with a pipette and allowed to act on hydrogen peroxide, using essentially the same proportions of all ingredients as specified for the basic procedure. The values obtained are shown in Table IV.

Table IV shows that a relatively small quantity of flour catalase is insoluble in the buffer solution, and that the amount is fairly constant regardless of the total catalase activity of the flour. For flours of very low catalase activity, this quantity represents nearly half of the total catalase, but for those of high activity it represents only 10% to 20% of the total. As to *soluble* catalase activity values, the flours stand in essentially the same relation to each other as in the case of total catalase values.

The data in Table IV suggest that if there is any general relationship between flour catalase activity and flour grade that will hold for all flours—regardless of type or origin—it is most likely to exist in the "insoluble" or "alpha" fraction. These values are, however, so small that unless a method of extraordinary precision can be developed,

TABLE IV

SOLUBLE AND INSOLUBLE CATALASE

Flour No.	Catalase by basic procedure	Catalase in extract alone	Difference (insoluble catalase)	Ratio soluble to insoluble
		mm. of pressure		
5	52	43	9	4.8
6	118	101	17	6.0
9	137	123	14	8.8
10	137	120	17	7.1
12	32	22	10	2.2
14	110	100	10	10.0
20	36	23	13	1.9
21	34	23	11	2.1
23	34	24	10	2.4
24	59	45	14	3.2
26	39	26	13	2.0
33	34	24	10	2.4

the probable errors involved in estimating differences among individual samples are likely to be nearly as large as the differences themselves. That such a method can be established and made of practical value seems somewhat doubtful.

Effect of Bleaching and Aging on Catalase Activity of Flour

A commercial clear flour showed a catalase value of 97 before bleaching, and after bleaching (with nitrogen trichloride and benzoyl peroxide) the value was 83. After standing one month at room temperature the value had dropped to 74. Natural aging alone appears to cause some appreciable reduction in catalase activity. Thus a clear flour dropped from 108 to 86 after two months' storage at room temperature. Under the same conditions, a patent flour dropped from 33 to 29.

Effect of Fine Grinding on Catalase Activity

Grinding for several days in a ball mill caused the catalase activity fo flour No. 6 to decrease from an initial value of 118 to a value of 33. The same treatment caused flour No. 11 to drop from 97 to 21. This is quite in agreement with the findings of Crocker and Harrington (1918), who noted that excessive pulverizing lowered catalase activity of seeds.

Summary and Conclusions

A simple manometric procedure for the measurement of catalase activity in flour is described.

Among flours of comparable grade, but of widely diversified origin, catalase activity varied widely and systematically with the regional or

climatic origin of the samples. Canadian flours had nearly four times as much catalase activity as flours from Texas or Kansas, intermediate regions having correspondingly intermediate flour catalase activity values. Wheat type or habit appears to affect catalase activity, spring wheat varieties showing substantially higher values than winter varieties grown under the same conditions. Among wheats of the same growth habits, varietal differences were not apparent.

Although the well-known parallelism between catalase activity and flour grade has occasionally been proposed as a basis for the ascertainment of degrees of flour refinement by means of catalase activity determinations, it is highly probable that such a procedure would have only limited application. Certainly a definite knowledge of the locality from which the wheat originated would be essential. It is also possible that different standards for different growth seasons would have to be established, due to varying climatic conditions from year to year.

An effort was made to determine whether flour catalase activity is a single factor or the resultant of two or more factors, by subjecting several flours of different catalase activities to various "differential" treatments. Each treatment altered the catalase values of the flours, but did not substantially change the order of their *comparative* values. This is interpreted to indicate that flour catalase is essentially a single and not a multiple factor.

Flour catalase activity is significantly reduced by commercial bleaching agents, and by natural aging at room temperatures. It is greatly reduced by excessive grinding in a ball mill.

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ESTIMATION OF DIASTATIC STRENGTH

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The usual methods for estimating diastatic activity are more or less unsatisfactory. Lintner's procedure is sound in principle, but is inadequate for precise quantitative results. Some of the more recently developed methods are cumbersome and objectionable in that they call for the gravimetric measurement of sugar. The method reported here is simple and accurate, and has the advantage of employing a rapid volumetric procedure for determining the sugar formed from starch by enzyme action.

The investigation was limited in respect to the study of certain variables, such as time and temperature of digestion. Only one temperature, 37° C., was employed, and but a few variations in time of enzyme action were attempted. Moreover, the enzymatic materials worked with were limited in kind. The method was standardized with Takadiastase. The exactness of the results obtained with widely varying concentrations of diastase appeared, however, to warrant a description of the method. The procedure adopted is as follows:

Mix 10 g. of soluble starch with a little water and pour into 250 cc. of boiling water, heating at the boiling temperature being continued for a few minutes. Cool, add 20 cc. of normal acetate buffer solution of pH 4.8 and dilute to 500 cc. Add

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50 cc. of the starch solution to a 100 cc. volumetric flask and place the latter in a 37° C. (±0.1°) water bath. When the starch solution has come to the temperature of the bath (about 15 minutes), add 5 cc. of diastase—containing solution and allow digestion to proceed for exactly 1 hour. Enzyme action is stopped by the addition of 10 cc. of .25 *N* sodium hydroxide. The flask is then cooled to room temperature and filled to the mark with water. Fill a 50 cc. burette with the solution. Titrate against 5 cc. of diluted (1 + 1) Fehling's solution, the latter first being brought to boiling in a small Erlenmeyer flask. (To avoid heating the burette and its contents, a bent outlet tube may be fitted to it.) With the exception of a reduced quantity of Fehling's solution, the procedure followed in determining the sugar in the solution is essentially that described by Lane and Eynon.[2] The end-point is determined with the aid of 4 or 5 drops of a 1% solution of methylene blue added directly to the boiling solution when the blue color of the copper solution has almost disappeared. The blue color of methylene blue fades out sharply when the reduction of the cupric copper is complete. For precise results, a rapid incremental titration is first made to determine the approximate amount of sugar-containing solution necessary to reduce 5 cc. of the diluted Fehling's solution. Then all but 1 cc. of the amount thus determined is added to 5 cc. of a fresh lot of the diluted (1 + 1) copper solution before heating is started. Boil 2 minutes, add indicator and complete titration in 1 minute. The cubic centimeters of sugar solution used in the final titration = *Ta*.

A blank to determine reducing substances in the starch solution and enzyme preparation is made as follows: Measure 50 cc. of the buffered starch solution into a 100 cc. volumetric flask, add 10 cc. of .25 *N* alkali and then (not sooner) the same quantity of enzyme solution as used in the determination described above. Make to volume and proceed as before. The cubic centimeters of inactivated enzyme—starch solution required to reduce 5 cc. of a 1 + 1 Fehling's solution = *Tb*.

To make correction for the combined reducing power of the starch and enzyme solutions, as found in the blank determination, the following equation is employed:

$$\frac{Tb \times Ta}{Tb - Ta} = Tc \quad \text{(corrected titration)}$$

The diastatic value of the substance tested is then found by the equation

$$\frac{43 \times 100}{E\,(Tc - 2)} = \text{diastatic value}$$

where *E* = milligrams of enzyme preparation used in digesting 50 cc. of starch solution (*i.e.*, milligrams of original enzyme preparation per 5 cc. of the solution used to digest the starch as described above).

Discussion

A series of determinations with varying amounts of Takadiastase, carried out under the conditions described above, led to the results shown in Table I.

When the values for the "corrected titration" (*Tc*) were plotted against the reciprocals of the milligrams of Takadiastase, a practically straight line resulted. It was also found that the empirically derived equation, milligrams Takadiastase $= \frac{43}{Tc - 2}$, fitted well the experimental data.

In the course of an investigation of distase-containing materials it was found convenient to establish an arbitrary scale for diastatic

[2] Lane, J. Henry and Eynon, Lewis. Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. J. Soc. Chem. Ind., Trans., 42, part 2 (1923), 32–37T.

TABLE I

Experiments with Varying Quantities of Takadiastase

Milligrams of Takadiastase taken	Titration (Ta)	Blank titration (Tb)	Corrected titration (Tc) $\left(=\frac{Tb \times Ta}{Tb - Ta}\right)$	Calculated milligrams of Takadiastase $\left(=\frac{43}{Tc - 2}\right)$
7.0	7.10	51.6	8.24	6.89
5.58	8.30	55.2	9.78	5.53
5.0	8.95	57.4	10.60	5.00
4.0	10.50	58.2	12.81	3.98
3.0	12.80	59.4	16.30	3.01
2.0	16.70	62.5	22.80	2.07
1.0	25.70	63.4	43.20	1.04

strength. For comparative purposes, a particular sample of Takadiastase was given a value of 100. The equation then became diastatic strength $= \frac{43 \times 100}{E\,(Tc - 2)}$, where $E =$ milligrams of diastase—containing material and $Tc =$ corrected titration.

Experience proved that accurate results could be expected when the quantity of enzyme material used per determination was such that the sugar titration (Ta) lay within the range of 5 or 6 cc. to 35 or 40 cc.

Variation in the time factor showed that the same quantity of sugar was formed whether a given quantity of enzyme acted upon soluble starch (under the standard conditions) for 60 minutes, or whether twice the quantity of enzyme was allowed to act for 30 minutes. In other words, enzyme concentration x time = a constant, appeared to be a very close approximation. But for a given time interval, relatively less starch hydrolysis occurred as the enzyme concentration increased. Thus doubling the enzyme concentration resulted in somewhat less than twice as much sugar formation. This effect may be observed by examination of Table III where it will be seen that 25 mg. of enzyme substance produced more than one-half as much sugar as 50 mg. acting under identical conditions of temperature, pH, etc. If exactly one-half the quantity of sugar had been formed with 25 mg. of diastase-containing material, the "corrected titration" (Tc) would have been 15.5 cc. whereas 14.63 cc. was found. Table II illustrates the first point mentioned, *i.e.*, enzyme concentration x time = a constant. In these determinations an enzyme preparation derived from a fungus growth was employed. The conditions were those obtaining in the standard procedure with the exception of reduced time in the first three.

A few tests showed that the material under investigation, which was an enzyme complex derived from the mycelium of a fungus, was most active diastatically at pH 4.8–5.0. This is also the hydrogen-ion con-

TABLE II

Variation in Time Factor

Milligrams of enzyme preparation 9b	Minutes	Corrected Titration (Tc), cc.
50.0	30	7.75
25.0	30	13.80
12.5	30	25.10
25.0	60	7.73
12.5	60	13.72
6.25	60	25.00

centration usually reported as being optimum for Takadiastase. The results shown in Table III were obtained with preparation 9b under conditions of varying hydrogen-ion concentration. Otherwise the standard procedure was followed.

TABLE III

Effect of pH on Diastase Activity

Milligrams of preparation 9b	pH of starch solution	Corrected titration (Tc), cc.
50	4.68	8.04
50	4.84	7.75
50	5.02	7.75
50	5.20	7.88
25	4.84	14.63

Since the quantity of sugar formed was inversely proportional to the titration values (Tc), it is evident from the above table that the optimum pH range was 4.8–5.0.

The experimental work done to develop the method, fix the limits of concentrations, time and pH values, was mostly that outlined above, but its use in the routine assay of diastatic activity of extracts of fungus growth was quite extensive and satisfactory.

While the equation milligrams of enzyme material $= \frac{43}{Tc - 2}$ appeared to hold equally well for Takadiastase and enzyme products similar to it prepared in the laboratory from certain fungi growths, the accuracy of its application in the estimation of malt and animal diastase would necessarily need to be checked. Any desired value could, of course, be substituted for 100 in the empirical equation given under the standard method.

AN IMPROVED APPARATUS FOR MEASURING GAS PRODUCTION AND EXPANSION IN DOUGHS

ROY IRVIN [1]

(Received for publication August 31, 1934)

Markley and Bailey (1932) called attention to the need for a gasometer for measuring under constant pressure the expansion and gas leakage of dough.

They devised a rather cumbersome apparatus to accomplish this result, constant pressure being maintained by means of a moving syphon arm attached to a float. A recent description of "Mariotte's bottle" (McCarthy, 1934), an apparatus for maintaining a constant rate of flow of liquid through a syphon, led the writer to apply the principle of this contrivance to a simpler and very satisfactory constant-pressure gasometer, that is quite sensitive, inexpensive, and free from moving parts.

"Mariotte's bottle" is shown in Figure 1. The short arm of the syphon *S* dips in the liquid in the closed vessel *A*, and air enters through the lower end of the tube *B* when the syphon is in operation. The rate of flow of liquid through the syphon is governed solely by the difference in level *H* between the lower end of the tube *B* and the outlet arm of the syphon. It is entirely independent of the level of the liquid in the vessel *A* as long as the end of *B* is below the liquid surface.

It was obvious that reducing the distance *H* to zero would stop the flow of liquid, and also that, *H* being zero, a slight increase in pressure in the tube *B* would cause liquid to flow through the syphon. The flow would cease, of course, if the pressure applied to *B* again became atmospheric.

The application of the principle to a constant-pressure gasometer is shown in Figure 2. *A* is a container for the dough, or other fermenting material, *B* is a reservoir of liquid to be displaced by gas from *A*, *S* a syphon, and *C* a graduated cylinder to collect the liquid forced out of *B*. To start the syphon, suction may be applied to the outlet arm *E*. When the tube *D* is filled with gas and the syphon *S* with liquid, no flow occurs if the lower ends of *D* and *E* are at the same level and the pressure in *A* is atmospheric. Increase in pressure in *A* will then cause liquid to flow from *E* as long as the pressure is applied.

[1] Industrial Fellow, Mellon Institute of Industrial Research, Pittsburgh, Pa.

In testing the operation of the apparatus, gas was generated in vessel *A* and a manometer attached to it as shown in Figure 2. The outlet arm *E* of the syphon *S* was alternately slightly raised or lowered from the level of the lower end of the tube *D*. Raising *E* caused increase in pressure in *A* and lowering it brought about a reduction in pressure.

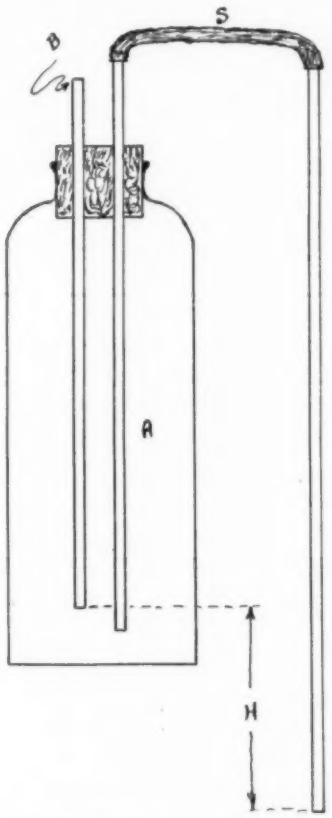

Fig. 1. Section of Mariotte's Bottle.
A—Closed bottle containing liquid.
B—Air inlet tube.
S—Syphon.
H—Head controlling rate of flow.

For any given position of *E*, the manometer showed no variation in pressure in *A* regardless of the changing liquid level in *B*.

To obtain constant atmospheric pressure in A, it was found necessary to fix the lower end of *E* slightly below the outlet *D*. The necessity for this adjustment could be explained by the capillary resistance of the syphon tubes.

It is, of course, obvious that the reservoir *B* should be of sufficient capacity to prevent the liquid in it dropping below *D* during a test.

In Markley and Bailey's gasometer the gas was collected over a film of oil floating on water to prevent absorption of carbon dioxide. In the apparatus described here the gas necessarily bubbles through the liquid in *B* (Figure 2), and to prevent its absorption a solution of calcium chloride, or other solution in which carbon dioxide is not soluble, might be used in *B*. (Jago (1911) recommended a calcium chloride solution

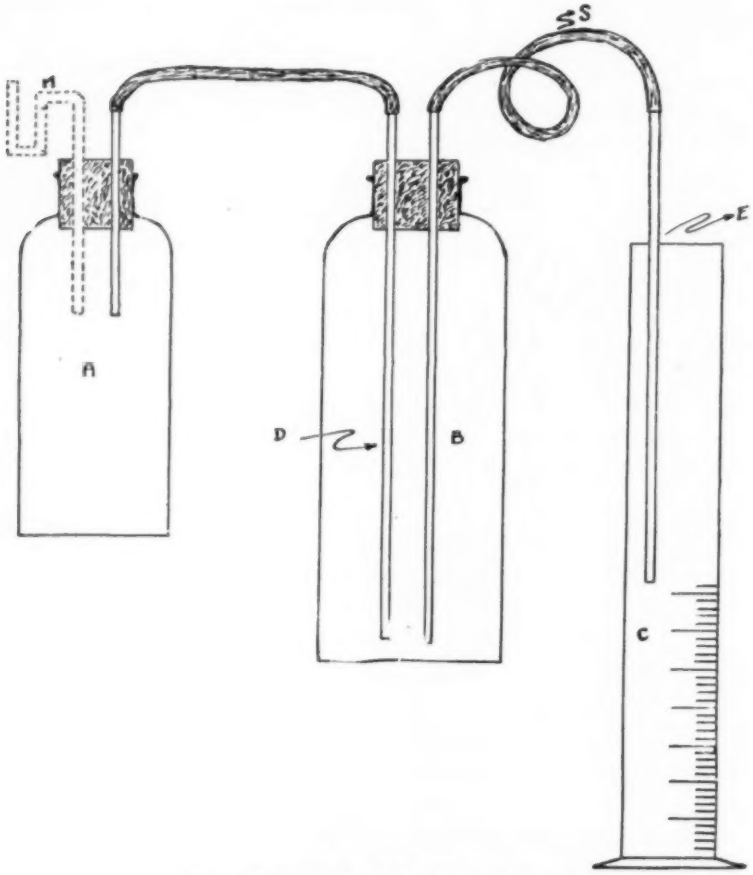

Fig. 2. Section of Constant Pressure Gasometer.
A—Container for dough.
B—Closed reservoir containing liquid in which carbon dioxide is not soluble.
C—Graduated cylinder.
D—Gas inlet tube.
E—Outlet arm of syphon.
M—Water manometer.
S—Adjustable syphon.

of 1.4 sp. gr. for this purpose.) Vessels *A* and *B* (Figure 2) should be placed in a controlled water bath to insure uniform and constant temperature in the parts of the apparatus containing gas.

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PEPTIZATION OF WHEAT FLOUR PROTEINS BY ORGANIC ACIDS [1, 2]

C. E. MANGELS and J. J. MARTIN, JR.

(Received for publication December 10, 1934)

Strong inorganic acids are recognized as reagents for precipitating proteins, and are also used for hydrolyzing proteins into their constituent amino acids. The complete hydrolysis of proteins, however, is only accomplished after prolonged boiling with strong acids.

Certain organic acids such as acetic acid are known to be excellent dispersing agents for proteins, but practically no information is found in the literature regarding the relative solvent or peptizing capacity of different organic acids, or the concentrations most suitable for use.

Vandevelde (1912a) digested yeast proteins with HCl and tartaric acids and concludes that the action of the two acids on yeast proteins is similar. Vandevelde (1912) also states that wheat gluten loses its elasticity when treated with tartaric, citric, or oxalic acids, but does not state what concentrations were used.

Blish and Sandstedt (1933) used 0.05 N acetic acid in combination with neutral salts and methyl alcohol to fractionate gluten proteins. They state that " Flour proteins dispersed in acetic acid alone, seemingly suffer no destructive changes in physical or chemical properties."

Gortner (1929) and associates have found wide differences in the peptizing capacity of neutral salt solutions for flour proteins. The present study was undertaken to determine if different organic acids would show similar variations in peptizing capacity.

Experimental

The flours used in this study were patent flours prepared in the experimental mill from wheats produced in the variety plots of the North Dakota Experiment Station, at Fargo, in 1933. The five flours represented five varieties of hard red spring wheat, namely, Marquis, Ceres, Reward, Marquillo, and Hope.

[1] Published with the approval of the Director as Paper No. 12, Journal Series, North Dakota Agricultural Experiment Station.
[2] Contribution from Department of Cereals and Milling, North Dakota Agricultural Experiment Station, Fargo, N. Dak.

The procedure used was briefly as follows: A 2 g. sample of flour was weighed into a 200 cc. Erlenmeyer flask and 100 cc. of the acid added from a pipette. The flask was stoppered and thoroughly shaken at 30-minute intervals for 4 hours, at room temperature. At the end of 4 hours, the flour suspension was centrifuged (or filtered) and a 50 cc. aliquot representing 1 g. of flour, placed in a Kjeldahl flask. Nitrogen was then determined on the aliquot by the usual procedure.

The organic acid extracts were with two exceptions, too viscous to filter and centrifuging was necessary. The extract with tri-chloracetic acid filtered readily and the same was true for the HCl and H_2SO_4 extracts. Extracts with H_3PO_4 and oxalic acids were filterable with difficulty, and the oxalic acid extracts were in all cases centrifuged.

Total nitrogen was determined on the flour and results were reported as percentage of the total N soluble in or peptized by the respective acid.

Effect of Acid Concentration on Peptization

Five comparatively common organic acids, namely, acetic, lactic, oxalic, tartaric, and citric acid, were used for this study. In this group of acids are represented mono-basic, di-basic, and tri-basic acids, and, also, three hydroxy acids. Concentrations of 0.10, 0.50, 1.0, and 2.0 normal were used. The data are given in Table I and shown graphically in Figure 1.

TABLE I

PROTEIN PEPTIZED BY ORGANIC ACIDS

Comparison of different concentrations

Acid	Total protein	Percent of total N peptized by			
		0.10 N	0.50 N	1.0 N	2.0 N
	%	%	%	%	%
Acetic [1]	12.4	84.1	85.2	83.9	79.5
Oxalic [1]	12.4	73.8	39.6	32.0	36.0
Lactic [1]	12.4	84.9	75.5	81.0	83.1
Tartaric [1]	12.4	84.5	76.5	76.5	75.4
Citric [1]	12.4	84.9	75.4	75.1	74.9

[1] Average of 5 varieties.

With the exception of acetic acid the greatest solvent or peptizing capacity was found at a 0.10 N concentration. With acetic acid, the 0.50 N concentration peptized 85.2% of the total N as compared with 84.1% for the 0.10 N acid. As the concentration of acetic acid was increased to 1.0 and 2.0 N the peptizing capacity decreased and at a 2.0 N concentration only 79.5% of the total N was peptized.

With lactic, tartaric, and citric acids, the solvent capacity showed a substantial decrease when the concentration was increased from 0.10 N to 0.50 N. Further increase in concentration did not significantly change

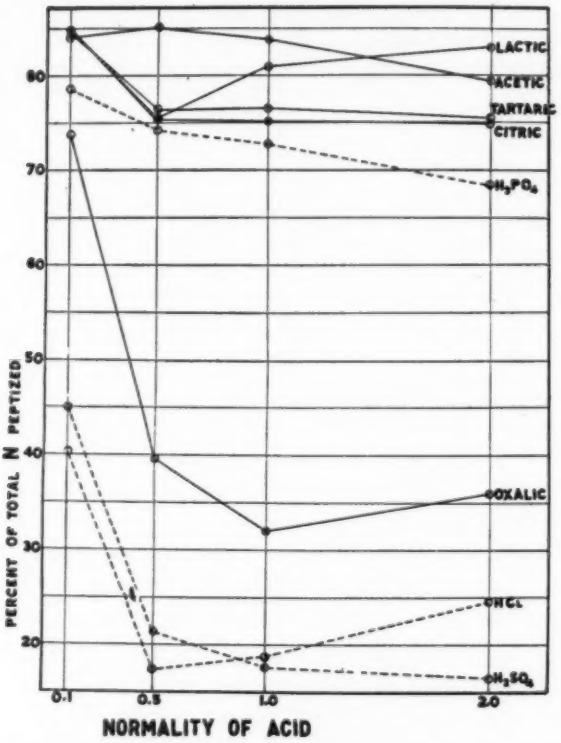

Fig. 1. Effect of concentration of acid on peptization of flour proteins.

the peptizing capacity of either tartaric or citric acid, but lactic acid showed an increase in peptizing capacity as its concentration was increased from 0.50 N to 2.0 N.

The 0.10 N oxalic acid peptized only 73.8% of the total N of the flour as compared with an average of approximately 85% for the other four organic acids. When the concentration was increased to 0.50 N only 39.6% of the total nitrogen was peptized, decreasing further to 32.0% with 1.0 N acid. With 2.0 N oxalic there was a slight increase, to 36%. The peptizing capacity of oxalic acid was lower at all concentrations than that of the other four organic acids used. Oxalic acid, in particular, showed a large decrease in dispersing power when the concentration was increased from 0.10 to 0.50 N.

Comparison with Inorganic Acids

It was deemed advisable to determine, for comparison, the effect of concentration on the dispersing or solvent capacity of inorganic acids. Three acids were selected, namely, HCl, H_2SO_4, and H_3PO_4. The results are given in Table II and also shown in Figure 1.

TABLE II

SOLUBILITY OR PEPTIZABILITY OF FLOUR PROTEINS IN INORGANIC ACIDS

Acid	% of total N—soluble or peptized			
	0.10 N	0.50 N	1.0 N	2.0 N
	%	%	%	%
HCl[1]	40.3	17.4	18.8	29.7
H_2SO_4[1]	45.0	21.4	17.7	16.5
H_3PO_4[1]	78.6	74.2	72.8	68.5

[1] Average of 5 varieties.

At a 0.10 N concentration hydrochloric acid dissolved or peptized 40.3% of the total N as compared with 45% and 78.6%, respectively, for sulphuric and phosphoric acids. As the concentration of HCl and H_2SO_4 was increased to 0.50 N the percentage of total N peptized decreased to 17.4% and 21.4%, respectively. When concentration was further increased to 1.0 N and 2.0 N, the peptizing capacity of H_2SO_4 showed a further decrease. Increasing the concentration of HCl to 1.0 N and 2.0 N, however, increased the solvent power of the acid. Since strong acids precipitate and denaturize proteins the consistent decrease in solvent power of H_2SO_4 is to be expected. With HCl, however, sufficient hydrolysis probably occurred to cause an apparent increase in solvent power, and this is not unexpected since HCl is generally regarded as a more efficient hydrolyzing agent for proteins than H_2SO_4.

Phosphoric acid at 0.10 N concentration peptized about 5% more of the total N than did oxalic acid. With increasing concentration, the solvent power of H_3PO_4 consistently decreased.

Phosphoric acid showed a peptizing capacity similar to the organic acids, and the effect of increasing concentration was also similar to that shown by organic acids.

The peptizing or solvent power of the inorganic acids at 0.10 N concentration varied inversely as their H-ion concentration. Since the solvent or peptizing action with 0.10 N inorganic acids varied inversely as their strength or H-ion concentration, it was considered advisable to compare the dissociation constants of some of the organic acids used. These data are given in Table III. Oxalic acid has a relatively high dissociation constant as compared with the other four organic acids and this apparently will account in part for the low peptizing capacity of this acid. Phosphoric acid peptized more protein than oxalic acid and this might be predicted from the dissociation constant, if H-ion concentration is regarded as the principal controlling factor. The dissociation constants of the other organic acids used are not of high order. Lactic acid (Figure 1) showed an increase in solvent power as concentration increases from 0.50 N to 2.0 N, while acetic acid decreased in

TABLE III

DISSOCIATION CONSTANTS OF ACIDS USED

Acid	Formula	
Hydrochloric	HCl	not well defined
Sulphuric	H_2SO_4	$*4.5 \times 10^{-1}$
Phosphoric	H_3PO_4	$*9 \times 10^{-3}$
Acetic	$CH_3 \cdot COOH$	1.8×10^{-5}
Oxalic	$COOH \cdot COOH$	$*3.8 \times 10^{-2}$
Lactic	$CH_3 \cdot CHOH \cdot COOH$	1.4×10^{-4}
Tartaric	$COOH \cdot (CHOH)_2 \cdot COOH$	$*1.1 \times 10^{-3}$
Citric	$COOH \cdot CH_2 \cdot C(OH)(COOH) \cdot CH_2 \cdot COOH$	$*8.0 \times 10^{-4}$
Propionic	$CH_3 \cdot CH_2 \cdot COOH$	1.4×10^{-5}
Butyric	$CH_3 \cdot (CH_2)_2 \cdot COOH$	1.5×10^{-5}
Valeric	$CH_3 \cdot (CH_2)_3 \cdot COOH$	1.6×10^{-5}
Succinic	$COOH \cdot (CH_2)_2 \cdot COOH$	$*6.6 \times 10^{-5}$
Mono-chloracetic	$CH_2Cl \cdot COOH$	1.55×10^{-3}
Tri-chloracetic	$CCl_3 \cdot COOH$	3×10^{-1}

* First step or first hydrogen only. Data from Landolt-Bornstein "Tabellen" (1912).

solvent power. The H-ion concentration may offer some explanation of this behavior.

In Table IV are compared the peptizing capacity of three acetic acids. The dissociation constants for these acids varied from 1.8×10^{-5} for normal acetic to 3×10^{-1} for tri-chloracetic. For the 0.10 N concentration, the peptizing capacity varied inversely as the dissociation constant or H-ion concentration. Tri-chloracetic acid is used frequently as a precipitating agent for proteins and these data show that it is superior as a precipitating reagent to either HCl or H_2SO_4 at the same concentration.

TABLE IV

PROTEIN PEPTIZED BY ORGANIC ACIDS

Comparison of acetic acids

	Total protein	Percent of total N peptized by		
		N/10 acetic acid	N/10 mono-chlor-acetic acid	N/10 tri-chlor-acetic acid
	%	%	%	%
Marquis	11.8	84.8	76.2	4.3
Ceres	12.7	85.8	76.0	3.5
Reward	13.8	84.0	70.4	3.8
Marquillo	12.2	82.7	72.3	4.5
Hope	12.3	83.0	72.5	4.2
Average	12.4	84.1	73.5	4.1

A rather different comparison is shown in Table V. The four acids, acetic, propionic, butyric, and valeric, differ progressively by the number of CH_2 groups. From Table III it will be noted that the dissociation constants for these four acids are all of the same order of magnitude, with acetic acid slightly higher. Acetic acid in this case showed considerably higher solvent power or peptizing capacity for flour proteins than propionic, butyric, or valeric acid. The average with the five varieties for acetic acid was 84.1%, while with propionic acid the average was only 73.6%. The further addition of CH_2 groups in butyric and valeric acids does not greatly affect peptizing capacity. The difference observed between acetic acid and its homologues cannot be ascribed to H-ion concentration.

TABLE V

PROTEIN PEPTIZED BY ORGANIC ACIDS

Comparison of mono-basic acids

	Total protein	Percent of total N peptized by			
		N/10 acetic	N/10 propionic	N/10 butyric	N/10 valeric
	%	%	%	%	%
Marquis	11.8	84.8	75.5	75.7	73.8
Ceres	12.7	85.8	75.2	75.2	74.1
Reward	13.8	84.0	73.4	73.6	71.8
Marquillo	12.2	82.7	71.9	71.9	70.3
Hope	12.3	83.0	72.0	72.7	70.7
Average	12.4	84.1	73.6	73.8	72.1

Comparison of Normal and Hydroxy Acids

Another comparison is shown in Table VI. The two hydroxy acids (lactic and tartaric) showed a higher peptizing or solvent capacity for

TABLE VI

PROTEIN PEPTIZED BY ORGANIC ACIDS

Comparison of normal and hydroxy acids

	Total protein	Percent of total N peptized by			
		N/10 propionic $CH_3 \cdot CH_2 \cdot COOH$	N/10 lactic $CH_3 \cdot CHOH \cdot COOH$	N/10 succinic $\begin{matrix} CH_2—CHOH \\ \vert \\ CH_2—COOH \end{matrix}$	N/10 tartaric $\begin{matrix} CHOH—COOH \\ \vert \\ CHOH—COOH \end{matrix}$
	%	%	%	%	%
Marquis	11.8	75.5	86.1	76.2	87.7
Ceres	12.7	75.2	85.8	76.7	85.4
Reward	13.8	73.4	85.6	74.6	84.4
Marquillo	12.2	71.9	83.0	72.8	82.0
Hope	12.3	72.0	83.9	73.1	83.1
Average	12.4	73.6	84.9	74.7	84.5

flour proteins than did their homologues, propionic and succinic acids. This was not surprising since the OH group was known to confer solubility and solvent power in organic compounds. The dissociation constants of the hydroxy acids were, in both cases, higher than the constants for their homologues, propionic and succinic acids, and the difference in peptizing capacity cannot be ascribed therefore to H-ion concentration.

Discussion

At least three factors, therefore, affect the peptizing capacity or solvent power of organic acids for flour proteins, namely, (1) H-ion concentration, (2) number of carbon atoms, and (3) presence or absence of hydroxy groups.

The pH of the fourteen 0.10 N acids used was calculated from their dissociation constants and compared with the average percentage of total N dispersed or peptized. Correlation by method of ranks gave a coefficient of + 0.4044 indicating that the peptizing capacity of the acids varied directly as the pH or inversely as the H-ion concentration.

H-ion concentration, however, is only one factor. A 0.10 N HCl solution (pH 1.0) dispersed an average of 40.3% of the total N, but 0.10 N tri-chloracetic acid (pH 1.1) dispersed only 4.1% of the total N. A 0.10 N H_2SO_4 solution (pH 1.1) dispersed an average of 45.0% of the total N as compared with an average of 68.5% dispersed by 2.0 N phosphoric acid (pH 1.1).

A comparison of the peptizing capacity of acetic and propionic acids showed that the introduction of a CH_2 group into the molecule decreased the dispersing power. A progressive decrease with addition of CH_2 groups does not occur, however, as evidenced by the similarity in peptizing capacity of propionic, butyric, and valeric acids. The higher dispersing capacity of acetic acid, therefore, appears to be due to the direct linkage of the CH_3 group to the COOH group.

The presence of OH groups increased the dispersing power of the acid, as evidenced by the comparison of propionic and succinic acid with lactic and tartaric acids. The OH groups in the three hydroxy acids used are in all cases in the α-position in respect to the COOH group.

Four of the organic acids used, acetic, lactic, tartaric, and citric, at 0.10 N concentration peptized approximately 85% of the total N. All other organic acids (with exception of tri-chloracetic) at the same concentration peptized 70 to 75% of the total N. Phosphoric acid peptized 75 to 80% of the total N and was therefore intermediate between the two groups of organic acids.

TABLE VII

PROTEINS PEPTIZED BY ORGANIC ACIDS

Varietal Variation

Acid	Percent of total N peptized				
	Marquis	Ceres	Reward	Marquillo	Hope
	%	%	%	%	%
N/10 acetic	84.8	85.8	84.0	82.7	83.0
N/10 propionic	75.5	75.2	73.4	71.9	72.0
N/10 butyric	75.7	75.2	73.6	71.9	72.7
N/10 valeric	73.8	74.1	71.8	70.3	70.7
Average Mono-basic acids	77.5	77.6	75.7	74.2	74.6
N/10 oxalic	75.9	75.1	73.7	71.6	72.8
N/10 succinic	76.2	76.7	74.6	72.8	73.1
Average Di-basic acids	76.1	75.9	74.2	72.2	73.0
N/10 lactic	86.1	85.8	85.6	83.0	83.9
N/10 tartaric	87.7	85.4	84.4	82.0	83.1
N/10 citric	86.7	86.2	84.2	80.4	87.2
Average Hydroxy acids	86.8	85.8	84.7	81.8	84.7

Varietal and Regional Variation

Table VII records the varietal variation in peptization by organic acids for the five varieties used in this study. Marquis and Ceres showed the highest percentage of protein peptized by organic acids and Marquillo the lowest. The order for mono-basic acids is Ceres, Marquis, Reward, Hope, Marquillo; and for hydroxy acids is Marquis, Ceres, Reward, Hope, and Marquillo. With neutral salt solutions used as peptizing agents, one of the authors (Mangels, 1934) found Marquis and Ceres most resistant to peptization but with organic acids as peptizing agents the order is reversed.

The variation between varieties is about of the same order or magnitude as that for neutral salt solutions (see Mangels, 1934), but the variation between varieties in this case is more consistent.

Table VIII records regional variations. The data presented are the average for the nine varieties grown at both Fargo and Dickinson. The Dickinson samples average consistently lower in peptizable protein with the five acids used. This is again the reverse of the results obtained with neutral salt solutions (Mangels, 1934) since the Fargo wheats were more resistant than Dickinson grown wheats, when salt solutions were used as peptizing agents. Varietal and regional variation are about the same order of magnitude for acetic, lactic, and oxalic acids, but with tartaric and citric acids varietal variation is larger.

TABLE VIII

PROTEINS PEPTIZED BY ORGANIC ACIDS

Regional Variation

Acid	Percent of total N peptized	
	Grown at Fargo [1]	Grown at Dickinson [1]
	%	%
N/10 acetic	84.1	81.2
N/10 lactic	84.8	81.0
N/10 oxalic	74.6	70.8
N/10 tartaric	85.0	81.7
N/10 citric	85.0	81.1

[1] Average of nine varieties.

Summary

The peptizing or dispersing power of five organic acids for flour proteins was determined using concentrations of 0.10, 0.50, 1.0, and 2.0 normal. With the exception of acetic acid the 0.10 N acids peptized more protein than the higher concentrations. The effect of increasing concentration varied with different acids.

Oxalic acid had a lower peptizing or dispersing power at all concentrations, than the other four organic acids used.

HCl and H_2SO_4 dispersed a much lower percentage of the total N than did the organic acids, but H_3PO_4 was similar to organic acids.

H-ion concentration was one factor which affected the dispersing power of organic acids for proteins. The dispersing power tended to vary inversely as the H-ion concentration.

Propionic, butyric, and valeric acids were similar in peptizing or dispersing capacity and lower in dispersing power than acetic acid.

Hydroxy groups increased the peptizing capacity of organic acids.

Regional and varietal variation in the peptizability of flour proteins was of similar magnitude to that obtained with neutral salt solutions. Varieties which showed a high resistance to the peptizing action of neutral salt solutions, were most susceptible to peptization by organic acids.

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REPORT OF THE 1933–34 PIE FLOUR COMMITTEE

C. B. KRESS, *Chairman*

Sperry Flour Company, San Francisco, California

(Read at the annual meeting, June 1934)

The purpose of the collaborative study was to determine a proper procedure for testing pie flours and to see if such a scheme was workable and would give proper information to the manufacturer and user of pie flour. The committee included members who were large manufacturers of pies and also those engaged in making pie flour. This branch of our particular division of flour testing is possibly a little more backward than bread flour testing, and we hope that this and future collaborative work will advance pie flour testing to the same standing as bread flour testing. Although this committee started their work in November, it found the time too short to conclude its program due to the great distance between collaborators and the fact that all contacts had to be made by correspondence. After a round robin for ideas, a complete testing procedure which incorporated the best ideas of each member of the committee was decided on. Twelve flour samples were sent to each member of the committee. The procedures used by the committee in carrying out the pie flour testing studies follow:

Directions for Collaborative Studies on Pie Flours

CHEMICAL DETERMINATIONS

Protein: Basis 13½% moisture.
Ash: Basis 13½% moisture.
Granulation: Use 10, 12, and 14XX bolting silk.
Color: A slick test.
Viscosity: 20 g. flour (basis 13½% moisture), 100 cc. distilled water, room temperature 68° F. Use mortar and pestle. Add 40 cc. of water to all of flour in mortar and work to smooth mixture, adding remainder of water while mixing until a smooth suspension is obtained. Transfer to viscosimeter and read at once. Then add 1 cc. normal lactic acid, stir and test again. Repeat after adding 2 cc. more lactic acid; again after adding 2 cc. more lactic acid, and again after 2 cc. more lactic acid, giving readings at 0, 1, 3, 5 and 7 cc. lactic acid. Report viscosity results, for example, as follows: 15, 25, 30, 32 and 32.

With MacMichael viscosimeter run at 12 r.p.m. use No. 30 wire and large bob. With Wallace & Tiernan viscosimeter use regular equipment for flour.

pH: Test pH of flour either electrometrically or colorimetrically.

BREAD BAKING TEST

Use A. A. C. C. formula (flour 100 g., yeast 3 g., salt 1 g., sugar 2½ g.).

Mix at 82° F. Ferment at 82° F., and make two tests. Use the following dough times and fermentation periods:

	A *3-hour dough*	*B* *4-hour dough*
First punch	105 min.	140 min.
Second punch	50 "	65 "
Pan	25 "	35 "

Proof about 60 minutes; bake about 25 minutes; measure volume of loaf in cubic centimeters; score crumb color, texture, grain, crust color, oven spring, and break down.

Pie Crust Baking Procedure

Formula

2½ lbs. flour	—or 150 g.	
1½ lbs. shortening	—or 90 "	(60%)
1¼ oz. salt	—or 5 "	
1 pint water at 42° F.		

Method

Put all the materials, except shortening (keep at room temperature to prevent from becoming too hard and causing lumps), to be used in the refrigerator at about 50° F. for about two hours before using, including the flour and water.

Shortening.—Use Crisco, melting point about 95° F. (suggest the ordinary household Crisco). This shortening is mentioned merely because it is available everywhere.

Rub shortening and flour together until in pieces one-half inch diameter, then add water with salt dissolved in it. Mix lightly until a soft mass is reached—one minute with a fork or by hand in a bowl—and put in the refrigerator and let rest for four hours at about 50° F.

After the dough has stood in the refrigerator four hours, remove and roll it out to ⅛-inch thickness on a lightly floured cloth. Spread this over a 10-inch pie tin, press down tightly all over the tin and prick well with a fork to let the steam out. Press a pan of same size down on top of dough to hold dough down. Put about a 1-pound weight in top pan. Bake for 10 minutes at 425°–450° F. and then remove top pan. Continue baking for 10 minutes longer or until done.

The fully baked shell can be used to judge without filling. From this, items Nos. 1, 2, 3, 4, 5, and 6, under "judging pie crust" can be judged.

Make up another dough for a filled pie, but do not prick the surface of crust for this type of pie. Dough for both shells can be mixed in one mixing and then divided into two equal parts. The formula is enough for only one shell, so double for both shells.

Custard Filling for Pie

Milk	1 qt.
Eggs	6
Corn starch	½ oz.
Sugar	8 oz.
Salt	1/12 oz.
Flavoring—mace and vanilla	

Mix sugar, starch, and eggs well, then add milk and flavoring, and mix thoroughly. Use this to fill the shell as follows:

After baking 10 minutes remove the pie tin from the top of shell and bake 5 minutes longer. Then fill at once while in the oven. (This mix is enough filling for two pies.) Fill nearly to top of shell. Bake about 25 or 30 minutes after adding custard. The top of custard should be fairly firm. Remove from oven and let stand about 16 hours and judge how much the bottom crust has soaked. Naturally the crust will soak some, but the bottom should not be real soggy.

JUDGING PIE CRUST

Qualities to be desired—1. Well baked through.
2. No shrinkage from edge of pan.
3. Golden brown color.
4. Flaky.
5. Tender.
6. Crisp.
7. Will not turn soggy within a reasonable time.

EXAMPLE OF SCORING

1. Well baked through or soft, doughy.
2. Shrinks away from edge of pan little or much.
3. Describe color.
4. Flaky or compact—granular.
5. Tender or tough (qualified by very or slightly, etc.).
6. Crisp or soft.
7. Let stand 16 hours and note sogginess of the crust. Has filling completely penetrated crust? What is its condition?

The response of the committee to active collaborative work has been splendid, so much so that it is utterly impossible to print all of the analytical data accompanying the committee's report; a general summary of the findings of the committee must suffice.

Conclusions

There is much ground for hope that pie flour can be judged by the usual chemical analysis coupled with a standard baking test. The characteristics of a flour for pie purposes it is believed can be revealed by the above tests. Naturally, a very close examination for minute differences will probably require a pie crust baking test. We believe, however, that a close study of the chemical, physical, and baking characteristics of a flour, if properly interpreted, will harmonize with an actual pie baking test.

One great difficulty has been, and still is, that the baking test (bread) was not properly carried out, particularly by those interested in pie flour. Naturally, the baking test has reached the most perfection in the centers of bread flour production and use.

The committee is well satisfied with the method suggested for making the pie crust test with the exception of that for the filled pie—to judge sogginess we used a custard pie and the directions given made it a too severe test. If we were to do it again we would use a fruit pie and change the method of procedure some. So far as the other characteristics of pie crust are concerned it worked perfectly.

It is believed that pie flour testing can be done with an equal degree of accuracy and agreement as bread flour testing, and it is recommended that the Association continue collaborative work to the point where a definite and workable procedure, that will accurately evaluate a flour for commercial pie making purposes, will result.

REPORT OF THE 1933–34 COMMITTEE ON METHODS OF TESTING SELF-RISING FLOURS

H. G. WALTER, *Chairman*

Igleheart Brothers, Inc., Evansville, Indiana

(Presented at the annual meeting, June 1934)

Introduction

In undertaking to derive a set of tests for self-rising flours, it was decided not to include analytical determinations as items for study at the present time, as these are being covered by other committees of the A. A. C. C. and to some extent by the Association of Official Agricultural Chemists. As the gluten quality of self-rising flours can only be judged by baking tests, and bearing in mind the recommendations set forth by the 1933 Sub-Committee on Tests for Biscuit and Self-Rising Flours,[1] it appeared advisable to continue the work as already started. Accordingly, the development of the biscuit baking test was resumed.

Experimental

The first problem studied was that of absorption. The collaborators carried out a series of bakings in an endeavor to obtain agreement with respect to the amount of water to be designated as optimum by each collaborator when using a common lot of flour. The results failed to show satisfactory agreements and a meeting of the committee was called at which variables heretofore not discussed in this work were pointed out. Dough consistency was found to be one point on which the committee had varied, and by means of bakings carried out at the time of the meeting, the collaborators reached a conclusion as to the proper softness or consistency of a dough.

A second series of bakings was undertaken by collaborators *A* and *C* in which agreement was obtained in the absorption item as shown in Table I. Collaborator *B*, using a Hobart mixer, found it necessary to work out a separate mixing procedure in order to obtain a dough similar to that made on the KitchenAid as used by the other collaborators.

In Table II are shown the results on the specific volumes of biscuits determined in duplicate on successive days, each collaborator using the predetermined optimum absorption.

[1] Walter, H. G. Tests for biscuit and self-rising flours. Cereal Chem. 10: 635–641 (1933).

TABLE I

COLLABORATIVE RESULTS ON ABSORPTION OF SELF-RISING FLOUR

Collaborator	A				C			
Absorption, %	57.5	62.5	67.5	72.5	55.5	61.5	67.0	72.0
Number of bakes	4	5	6	6	6	6	6	6
Average specific volume [1]	1.707	1.736	1.850	1.820	1.708	1.765	1.788	1.757
Average error, %	±1.32	±2.67	±1.44	±1.83	±3.07	±1.98	±2.07	±2.03
Total score	85.2	90.7	95.0	92.4	88.7	93.1	95.4	94.3
Average error, %	±0.50	±1.65	±0.56	±0.72	±2.23	±1.15	±1.02	±1.24

[1] Basis weight of dough.

TABLE II

SPECIFIC VOLUME MEASUREMENTS ON DUPLICATE BAKINGS MADE AT OPTIMUM ABSORPTION ON SUCCESSIVE DAYS

Collaborator	A	B	C
Specific volumes [1]			
First day	1.83	1.88	1.90
	1.82	1.82	1.77
Second day	1.82	1.90	1.74
	1.90	1.77	1.78
Third day	1.85	1.90	1.78
	1.88	1.83	1.76
Average specific volume	1.850	1.850	1.788
Average error, %	±1.44	±2.32	±2.07

[1] Basis weight of dough.

The formula and procedure used in this work are as follows:

FORMULA

Flour (self-rising) 15% moisture basis	210 g.
Hydrogenated shortening	26 g.
Distilled water	Variable
Temperature of dough	20° C.

The self-rising flour was prepared by the Chairman, using the committee's recipe which is 200 parts flour, 3 parts sodium bicarbonate, 3.75 parts monocalcium phosphate and 4 parts salt. The shortening was also sent out from one common lot.

Procedure: *For use with Kitchen Aid*

Sift flour twice and place in a 3 qt. mixing bowl. Add solid shortening and mix, using the flat beating paddle for two minutes at low speed. Add water and mix with paddle for 20 seconds at low speed.

Procedure: *For use with Hobart*

Sift flour twice and place in a 3 qt. mixing bowl. Break up shortening by hand with the paddle, mix 3 minutes at 1st speed, add water and mix 10 seconds at 2d speed.

Turn out the dough on a lightly floured, cloth-covered board between sticks, roll with a cloth-covered rolling-pin and fold double, re-roll and again fold double (this time at a right angle to the first roll), and re-roll between sticks or an embroidery hoop, and cut seven 2-inch biscuits ½ inch thick. The sticks or embroidery hoop should not be over ⅜ inch thick, otherwise the cut dough will exceed ½ inch thickness. Place 6 biscuits in a circle, one in the center, and use the remaining dough as a protective wall around the biscuits. Bake at 475° F. for 12 minutes.

Determine specific volume after cooling for ½ hour. Score, using the card shown in last year's report.[1]

Standardizing Items for Scoring

Volume: With respect to standardizing score items, work on volume, grain, and flavor have been taken up. The committee agreed on the arbitrary specific volume of 2.00 to which a value of 40 is given on the score card to denote volume. In determining the specific volume, the volume is measured by seed displacement, using apparatus standardized against a set of 7 dummies of known collective volume.

Grain: H. V. Moss, Provident Chemical Works, St. Louis, Mo., was assigned the problem of standardizing grain for scoring adaptability. A wide ash range of soft wheat flours was used, giving them a numerical grading. This procedure, however, was largely arbitrary and influenced by the experience of those judging the biscuits. Consideration was given to (1) cell size and shape, (2) cell uniformity, and (3) thickness of cell wall. As variations in the color of the flours affected the judging of grain, the biscuit sections were dyed with ordinary water soluble red writing ink. This proved to be the better coloring substance in covering up the difference in flour color.

It has been the experience of this collaborator that grain may only be evaluated by reference to a standard, and the judging of the test flours was done by resorting to this practice and by selecting standards from the flours tested. In order to arrive at some measure of agreement between laboratories it is evident that standards for grain must be established. It is believed that differences in grain may be faithfully portrayed in photographs of biscuit sections and that reference standards may be established by pictures of cross and/or vertical sections of flours of known properties.

Flavor: R. A. Barackman, Victor Chemical Works, Chicago, Ill., was assigned the problem of standardizing flavor as applied to our biscuit baking test. No elaborate summary of the scientific and trade journal literature on flavor appears necessary for the purpose of this report, but it is generally agreed that flavor is a combination of sensory effects imparted by the odor and taste of a baked product. A particular

effect observed by an individual may not be as pronounced to another, and moreover is to a large extent dependent on personal prejudice and inclinations. For judging flavor characteristics of a biscuit, the committee agreed that the following points are observable. These points, which are listed below, are, in brief, a summary of the literature on the subject plus the addition of points of particular interest to the self-rising flour industry.

Points for Consideration in Scoring Biscuits

	Desirable	*Undesirable*
Flour condition	sweet	rancid, musty
Flour quality	sweet	wheaty, starchy
Leavening	neutral	acid or sour, alkaline or soapy
Salt	pleasing	salty, flat
Eating quality	good or chewy	doughy, dry and crumbly

Foreign flavors as contaminating material or absorbed odors; foreign grain (oats, corn, etc.), straw mustiness, kerosene, pitch, phenols, camphor, garlic.

The committee favors scoring flavor on the hot biscuit and considers it desirable to increase the formula to obtain an extra biscuit. The extent of mark down for undesirable flavor will be left to the scorer and should be accompanied by a write-in or check-off system in conjunction with the numerical score using the undesirable features listed above. Thus the reasons for a reduced score will translate this figure into tangible faults so that a clear understanding will be conveyed with the report. In cases where no particular fault may be found with a self-rising flour, yet the biscuit as a whole is not up to a predetermined standard, the general information may be indicated by "very good," "good," "fair," "poor," "very poor."

Suggestions for Future Committees

Standards for tenderness and crumb color should be developed.

The study of grain evaluation should be continued and directed primarily toward the establishing of standards consisting of photographs of biscuits made from flours varying widely in inherent and leavening properties.

The proposed method of judging flavor should be practiced by future collaborators.

Collaborative bakings should be continued using the tentative method described above making use of scoring standards as they are developed, with a direct aim at reaching close results on one and the same flour, after which work may be taken up to test the accuracy of the baking test in differentiating between flours differing in quality.

Additional work paralleling that recorded in Table I should be undertaken by a larger number of laboratories to more definitely determine the measure of agreement in establishing optimum absorption.

THE AGING OF WHEAT FLOUR AND THE NATURE OF THIS PROCESS

NATALIE P. KOZMIN

Scientific Institute for Cereal Research, Moscow, U. S. S. R.

(Received for publication May 15, 1934)

The aim of the investigation reported here was to study the changes taking place in the quality of flour gluten during the process of the aging of flour and to examine the cause for these changes.

Six samples of freshly milled wheat flour were taken for the experiments. Three of these flours possessed strong elastic gluten, while the other three flours were characterized by typically weak gluten. The difference between these two types of flour (strong and weak) was especially and clearly marked after a three-hour standing of the washed gluten. The strong gluten remained elastic during this period, while the weak gluten lost its elasticity entirely and could be pulled out to any length.

All samples of flour were tightly packed into jars which were hermetically sealed. One series of samples was stored at 15° C., another at 30° C., and a third at 45° C., from one to three months.

The flour stored at the higher temperatures (30° C. and 45° C.) showed a very sharp change in gluten quality within two months. The strong flour gave, during washing, a very brittle, granular gluten which could not stand pulling at all. However, after standing three hours, the granular mass became more adhesive and turned into a compact, strong lump, hard to the pull.

The same flour stored at a low temperature showed almost no change in the quality of the gluten.

Observations taken during these investigations are shown in Figures 1, 2 and 3. In Figure 1 is shown (1) a photograph of the washed gluten from the strong flour after storage at 45° C. for two months; (2) illustrates the gluten of a similar flour freshly milled and photographed immediately after washing. On the left in Figure 2 is shown gluten freshly washed from aged flour; on the right, gluten from the same flour three hours after washing. Figure 3 illustrates, from left to right, the gluten of strong flour stored at a low temperature; the gluten from the same flour stored at a high temperature; and the gluten from the same flour, but freshly milled, photographed three hours after washing.

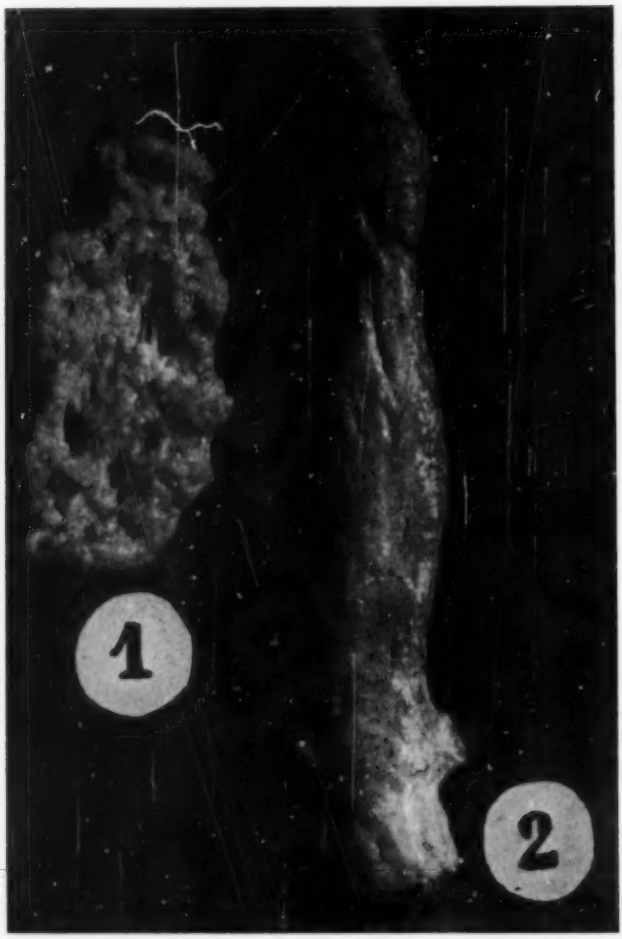

Fig. 1. Gluten of the aged flour (1) and gluten of freshly milled flour (2).

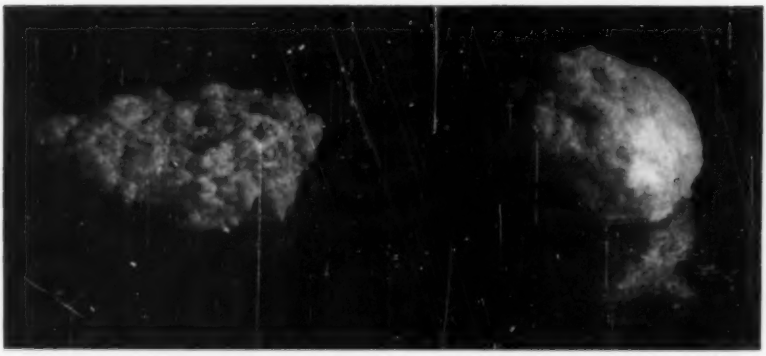

Fig. 2. Left—gluten from aged flour freshly washed. Right—gluten from same flour 3 hours after washing.

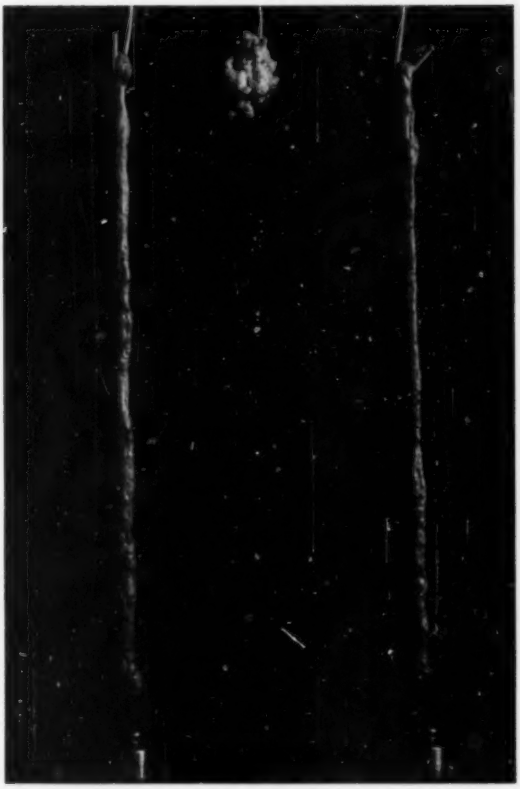

Fig. 3. Left, gluten of flour stored at 15° C. Middle, gluten of flour stored at 45° C. Right, gluten of freshly milled flour.

As the result of aging, the weak flour became nearer in gluten quality to that of the freshly milled strong flour. This is illustrated in Figure 4, exhibits 1 and 2, left to right: Gluten of weak flour stored at a low temperature; gluten of the same flour but stored at a high temperature, respectively. An explanation concerning the other two glutens shown in Figure 4, photographed three hours after washing, will be given below.

During the storage of flour at a high temperature there could be observed a strong rise of titratable acidity as well as an increase in the acid number of fat. The content of the free acids in the fat of this flour amounted to almost 50%.

The removal of fat from the aged flour by ether extraction returned the gluten to its original quality, making it " young " again. The effect of ether extraction upon the strong flour can be seen from Figure 5, where at the left is shown the gluten of the aged flour, and at the right the gluten of the same flour after the removal of fat.

Fig. 4. Gluten of weak flour.

The effect of ether extraction upon the aged weak flour is demonstrated in Figure 4, fourth figure from left.

The addition of fat from aged flour, or of additions of chemically pure acids of which flour fat is composed (oleic, for example), to freshly milled flours affects the gluten in exactly the same way as

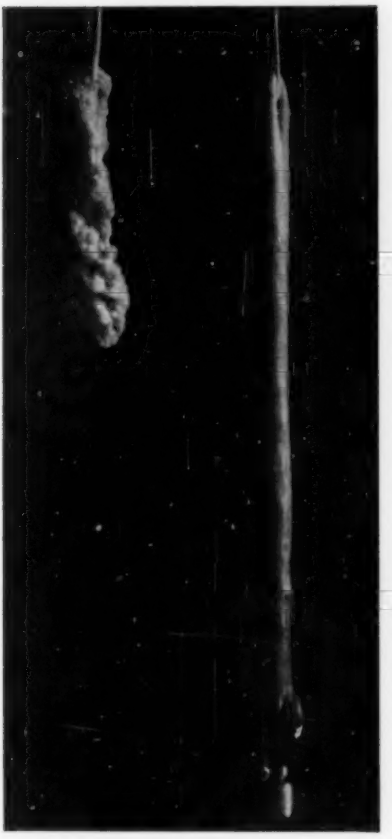

Fig. 5. Effect of fat extraction.

natural aging; the gluten of the strong flour becomes still stronger and more easily tearable, passing through to the stage of granularity. These effects are illustrated in Figure 6 where, left to right, the following are illustrated: Gluten of freshly milled flour; gluten of flour stored at a high temperature and gluten of the first flour to which 0.75% of oleic acid has been added, respectively, all photographed immediately after washing.

By the addition of oleic acid the gluten of the weak flour obtained considerable elasticity, becoming more like the strong flour (see Figure 4, third exhibit from left).

From the experiments carried out, the following conclusions can be drawn:

During the storage of flour a change in the quality of gluten in the direction of its strengthening has been observed.

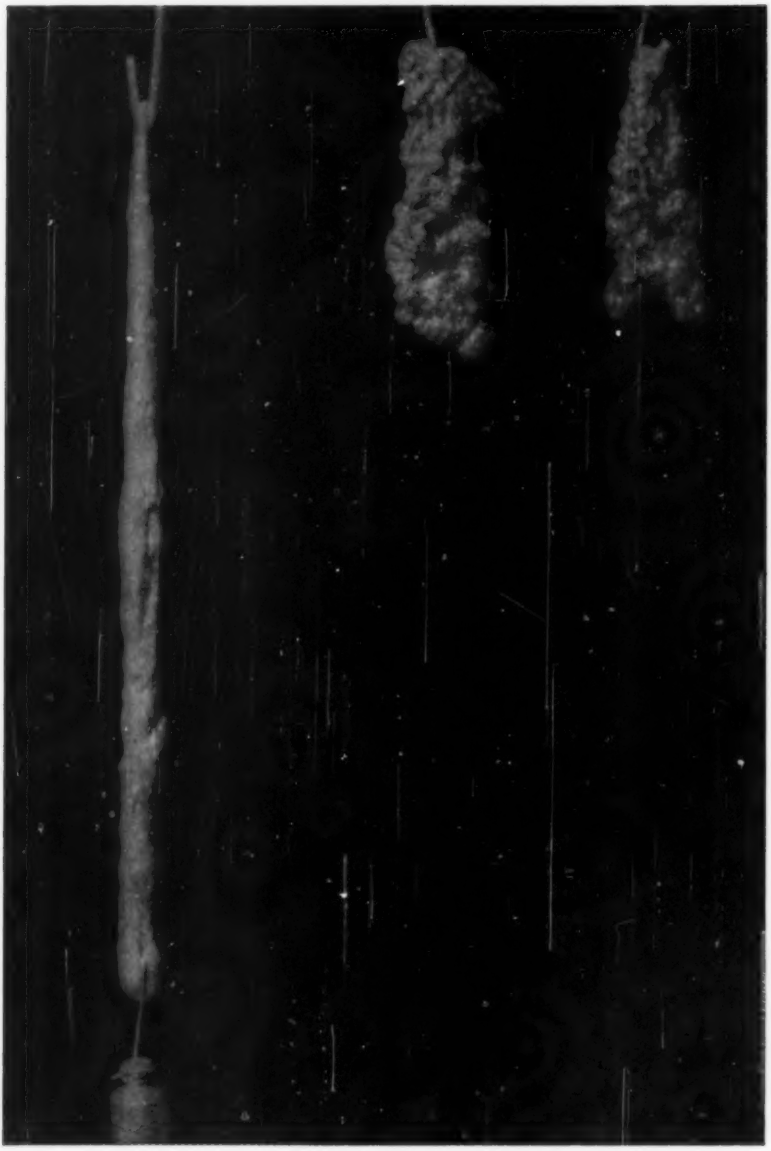

Fig. 6. Effect of aging and of addition of oleic acid.

The direct cause of this change is the accumulation of free fatty acids in the flour, caused by a slow process of fat hydrolysis.

The removal of fatty acids from the flour rejuvenates the latter, returning it to its original state.

In turn, the addition of free unsaturated fatty acids produces the same effect as natural aging.

Free unsaturated fatty acids (oleic, linoleic, linolenic, etc.) influence the colloid behavior of gluten in a specific manner, thickening the gel. Saturated fatty acids with the same amount of carbon atoms (stearinic, palmitinic, etc.) show no such effect.

The process of flour aging proceeds without participation of oxygen, as fat hydrolysis does not require the presence of the same. Oxidation of flour pigments taking place during aging has no connection with the change of gluten quality.

The chemical effect of artificial aging by means of bleaching is different from the natural aging and is probably connected with the action of the strong oxidizing agents upon the protein of the gluten.

Temperature is the most important factor influencing aging of the flour.

More complete data on these studies will be found in Das Muhlenlaboratorium B. 4, Heft 8, 1934, under the title " Kleberqualität und einige Faktoren die diese Beinflussen." N. Kozmin.

ANNUAL REPORT OF SECRETARY-TREASURER

M. D. Mize

January 1, 1935

DETAILED MEMBERSHIP STATEMENT DECEMBER 31, 1934

	Total	Active	Corp.	Hon.
Membership, Dec. 31, 1933	446	401	43	2
New members added during 1934	46	43	3	0
Members reinstated during 1934	8	7	1	0
Members resigned and suspended for non-payment of dues during 1934	*25*	*24*	*1*	0
Members deceased during 1934	*2*	*2*	0	0
Members in good standing Dec. 31, 1934	473	425	46	2
Net increase in membership during 1934	27	24	3	0

PROFIT AND LOSS STATEMENT

January 1 to December 31, 1934

RECEIPTS 1934			
Cereal Chemistry			
Membership dues			
Active		$1,494.50	
Corporation		460.00	
Subscriptions, reprints, back numbers and advertising	$3,764.20		
1934 Accounts Receivable	242.02		
1933 Income received in 1934	*45.40*		
Net 1934		3,960.82	
Interest on Invested Funds		85.50	
Total Net Receipts 1934			$6,000.82
Association			
Membership dues		1,487.50	
Application Fees		129.50	
Interest on Invested Funds		67.77	
Toronto Convention Registration Fee (A)		110.08	
Miscellaneous Income		25.50	
Total Net Receipts 1934			1,820.35
Cereal Laboratory Methods Reserve Fund			
Interest on Invested Funds			18.00
Convention Reserve Fund			
Interest on Invested Funds		18.00	
Balance of Toronto Registration Fee (B)		27.46	
Total Net Receipts 1934			45.46
Decennial Index			
Received from Cereal Chemistry and Association			*272.91*
TOTAL RECEIPTS OF ALL ACCOUNTS 1934			$8,157.54

DISBURSEMENTS

Cereal Chemistry			
Cost of printing Journal and Reprints	$4,575.08		
1934 Accounts Payable	806.34		
1933 Accounts Paid in 1934	*928.49*		
Net 1934		$4,452.93	
Cost of Editing and Miscellaneous Expenses	$1,376.18		
1934 Accounts Payable	91.02		
1933 Accounts Paid in 1934	*153.90*		
Net 1934		1,313.30	
1933 Accounts Uncollectable		39.14	
Decennial Index—Cereal Chemistry's 1934 Assessment		191.66	
Net Disbursements 1934			$5,997.03
Surplus 1934			$ 3.79
Association			
Expenses of President's, Vice-President's Office, News Letter		343.04	
Expenses of Secretary-Treasurer's Office		396.98	
Committee Expenses		36.20	
Toronto Convention Expenses		155.53	
Toronto Convention Report	$ 99.30		
Toronto Convention Report Accounts Payable	189.57		
		288.87	
Decennial Index—Association's 1934 Assessment		81.25	
Miscellaneous Expenses		37.50	
Net Disbursements 1934			1,339.37
Surplus 1934			480.98
Cereal Laboratory Methods Reserve Fund			
Mailing Expenses			7.50
Surplus 1934			10.50
Convention Reserve Fund			
Surplus 1934			45.46
Decennial Index			
Editing Expenses		200.00	
1934 Accounts Payable		560.41	
Net disbursements 1934			760.41
Deficit 1934			*487.50*
TOTAL DISBURSEMENTS OF ALL ACCOUNTS			$8,104.31

DISTRIBUTION OF NET ASSETS

Cereal Chemistry Assets 1933	$3,094.12	
Surplus 1934	3.79	
Assets Dec. 31, 1934		$3,097.91
Association Assets 1933	2,777.78	
Surplus 1934	480.98	
Assets Dec. 31, 1934		3,258.76

Cereal Laboratory Methods Reserve Fund 1933	451.98	
Surplus 1934	10.50	
Assets Dec. 31, 1934		462.48
Convention Reserve Fund 1933	598.02	
Surplus 1934	45.46	
Assets Dec. 31, 1934		643.48
Experimental Laboratory Baking Fund 1933	84.70	
Assets Dec. 31, 1934		84.70
Decennial Index Fund		
Deficit 1934	*487.50*	
Assets Dec. 31, 1934		*487.50*
TOTAL ASSETS DEC. 31, 1934		$7,059.83

FINANCIAL STATEMENT DECEMBER 31, 1934

U. S. National Bank—Checking Account	$ 205.19
Cash on Hand	187.27
Petty Cash Fund in Washington, D. C.	200.00
First National Bank—Savings Dept.	1,979.45
U. S. National Bank—Savings Dept.	18.27
Harris Trust Company—Savings Dept.	1,874.97
Building & Loan Stock in Kansas City	2,000.00
U. S. Treasury Bonds	2,000.00
1934 Income Receivable	242.02
GROSS ASSETS	8,707.17
LIABILITIES	
1934 Accounts Payable	*1,647.34*
NET ASSETS	$7,059.83

Note: All amounts in italics are negative amounts and are subtracted from the other amounts in the same column.

REPORT OF AUDITING COMMITTEE

H. H. JOHNSON, *Chairman*

The Auditing Committee has examined the books of the Secretary-Treasurer for the year 1934 and to our knowledge the financial report of the Secretary-Treasurer displays the true financial condition of the American Association of Cereal Chemists.